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Dietary Fat and Tumor Formation*†

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INTRODUCTION

Diets high in fat have been shown to accelerate the formation of mouse tumors produced by ultraviolet light (1) or by the local application of benzpyrene (2). This acceleration has been observed in several strains of mice on several basal rations, and with 3 hydrocarbons—3,4-benzpyrene, 20-methylcholanthrene and 1,2,5,6-dibenzanthracene (15). The action of increased dietary fat was unique; other variations in the diet failed to increase tumor formation. Since the application of hydrocarbon was continued on all diets until tumors appeared, the most obvious effect of fat was upon the rate of production rather than upon the number of tumors ultimately produced. However, carcinogenic hydrocarbons apparently need not be given throughout the precancerous period, for mouse tumors have been reported when hydrocarbons were applied for periods as short as 7 or 8 weeks (18, 19, 27). Hence, the possibility existed that a more intense effect of fat would be revealed if the carcinogen were administered for only a limited period. This proved to be the case. The new technic was accordingly used in studies on the effect (a) of various fat fractions, (b) of fats which had been mildly rancidified, and (c) of fats fed during various phases of the precancerous period. Finally, the relation of fat to tumor formation was investigated in another species of animal, the rat.

METHOD

The experimental procedure was essentially that reported in previous papers (2, 15). Tumors were produced in commercial albino mice of unknown genetic background by the application twice weekly of 0.2 per cent or 0.3 per cent solutions of methylcholanthrene in dioxan to a depilated area of the back. All applications were made by the same operator, and consisted of 2 uniform strokes of a freshly dipped camel's hair brush. Food and water were given ad libitum. The mice were examined twice monthly for tumors, and the results expressed as the per cent of the effective total [1] (10), which developed tumors. The experiments were performed in a number of series in each of which the animals were divided into groups of 25 comparable in age and weight. All groups within a series received the same amount of hydrocarbon, but each group received a different diet.

The basic ration was composed of the following ingredients:

Corn meal	445 gm.
Skim milk powder	222 gm.
Linseed oil meal	167 gm.
Soybean meal	111 gm.
Alfalfa meal (fine, green)	33 gm.
NaCl (fine, iodized, contained .023 per cent KI)	11 gm.
$Ca_3(PO_4)_2$	11 gm.

In each series 1 group of mice was fed a diet relatively low in fat, the "control diet." This consisted of: basal ration, 93 per cent; brewers' yeast, 5 per cent; and cod liver oil, 2 per cent. The other groups received experimental diets which were the same as the control diet except that fat or a derivative was substituted for an equivalent weight of the basal ration.

All results reported for mice were observed in at least 2 different series, and the series revealing the most significant results were repeated oftener. The results with rats, however, represent a single series. The procedure employed in the experiments with rats is described in a separate section.

EXPERIMENTAL

Minimum treatment with methylcholanthrene.—As a basis for the projected experiments it was necessary to ascertain what conditions were just insufficient for tumor production in mice receiving the control diet.

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[1] The effective total is defined as the number of animals alive in a group when the first tumor appears in that group.

These conditions were achieved when a 0.2 per cent solution of methylcholanthrene was applied twice weekly for 2 or 2½ months (Table I); tumor incidence at 6 months averaged 12 per cent or less. The procedure using borderline doses of carcinogen proved to be ideal for demonstrating the effect of fat in carcinogenesis. Thus, in a typical series, tumor incidence was 83 per cent at the end of 4 months when the diet contained 15 per cent of fat,[2] but only 6 per cent when the control diet was fed (Table II). In other words, the presence of dietary fat determined whether tumors would appear in a low or in a high percentage of the animals. The effect was shown repeatedly in series in which the survival of the animals was sufficient to give reliable results.

Table I: The Production of Tumors in the Skin of Mice with Minimum Amounts of Methylcholanthrene

Diet	Carcinogenic treatment	Percentage of mice with tumors at various months									Effective total
		2	2½	3	3½	4	4½	5	5½	6	
Control	0.2% MC,* 2½ months	0	6	6	6	6	6	6	6	6	15
Control	0.2% MC, 2 months	0	12	12	12	12	12	12	12	12	16
Control	0.3% MC, 2 months	0	0	5	11	11	16	16	22	..	18
Control	0.3% MC, 2 months	0	0	0	c	0	6	11	11	17	17

* Methylcholanthrene.

Table II: The Effect of Dietary Fat on Tumor Formation

Diet	Carcinogenic treatment	Percentage of mice with tumors at various months									Effective total
		2	2½	3	3½	4	4½	5	5½	6	
Control	0.2% MC,‡ 2 months	0	6	6	6	6	6	6	6	6	15
15% fat *	0.2% MC, 2 months	0	25	33	75	83	..	..	..	..	12
Control	0.3% MC, 2 months	0	0	5	11	11	16	16	22	..	18
15% fat †	0.3% MC, 2 months	0	28	78	93	93	..	..	..	..	14
Control	0.3% MC, 2 months	..	..	0	0	0	6	11	11	17	17
15% fat †	0.3% MC, 2 months	..	..	62	71	86	86	86	86	86	21

* Coconut oil.
† Hydrogenated vegetable oil.
‡ Methylcholanthrene.

The effect of fat fractions.—Some of the more distinctive components of fat were next investigated for their potency in stimulating tumor production. Animals receiving the borderline doses of carcinogen were fed diets containing the following: 5 per cent glycerol, 15 per cent ethyl laurate, or the unsaponifiable matter of hydrogenated vegetable oil [2] equivalent to 30 per cent of fat. Other groups in the series were fed the control diet and a diet containing 15 per cent of fat.[2]

The usual methods were employed to prepare the fat fractions. The glycerol was a commercial specimen which was redistilled under reduced pressure. The ethyl laurate was prepared from lauric acid [3] by esterification with 4 volumes of absolute ethyl alcohol in the presence of 0.08 volumes of concentrated H_2SO_4; the ester was extracted with ether, washed with 10 per cent Na_2CO_3 solution and distilled water, and the solvent removed under reduced pressure. The ethyl laurate was finally distilled through a fractionating column under reduced pressure at a temperature of 170-180° C. The unsaponifiable matter was prepared by saponifying hydrogenated vegetable oil [2] with alcoholic KOH, and extracting with ether; the ether extract was washed thoroughly with distilled water, and the solvent removed under reduced pressure.

The results of feeding these preparations are given in Table III. Each of the fat fractions caused some increase in tumor formation. However, ethyl laurate was the most effective; 63 per cent of the animals in this group had developed tumors at the end of 4 months, as compared with 42 per cent in the group on glycerol, and 35 per cent in the group on the unsaponifiable fraction. Furthermore, ethyl laurate proved to be just as effective as the untreated fat; *i.e.*, at the end of 4 months, the percentage of animals with tumors in these groups was 63 and 60 per cent, respectively. The relative ineffectiveness of glycerol and of the unsaponifiable matter was revealed when the amounts of these fractions were calculated as they occur in a diet containing 15 per cent of fat. The amount of glycerol equivalent to 15 per cent of fat is less than 2 per cent, whereas in the present series glycerol was fed at a level of 5 per cent. Similarly, the unsaponifiable matter was fed at a level twice that found in a diet containing 15 per cent of fat. It is evident therefore that the fatty acid fraction is largely responsible for the fat effect. Attempts to feed free fatty acids, such as oleic or lauric, or the mixed acids of hydrogenated cottonseed oil were, however, unsuccessful because the animals failed to survive long enough for tumor formation.

[2] This is sold under the trade name "Primex."

[3] The lauric acid was a product of the Lucidol Corporation, Buffalo, N. Y.

The effect of treated fat.—The prolonged feeding of heated fat or heated cholesterol has been reported to induce cancers in the intestinal tract of rats and the results interpreted to indicate the presence of a carcinogenic agent in the heated material (23, 24, 28). However, another possibility which might be entertained is that the heated material merely accelerated the action of natural carcinogens. This would constitute a reaction analogous to that of ordinary non-carcinogenic fat in mice painted with methylcholanthrene (15). In view of this, hydrogenated vegetable oil was subjected to the following procedures: the 1st sample, 300 gm., was spread into a thin layer and irradiated in air for 24 hours at a distance of 14 inches from a quartz mercury vapor lamp. A 2nd 300 gm. sample was maintained in a molten condition at a temperature of 45° C. by means of a 100 watt electric lamp, while air was bubbled through it until peroxide values of 50 to 65 were obtained. The reaction was activated by the addition of 0.005 per cent copper oleate. A 3rd sample, 300 grams, was heated in an open glass container to a temperature of approximately 300° C. The heat treatment was continued for 1 hour, 30 minutes of which was required to attain the maximum temperature. The samples were stored in the refrigerator until ready for use, and fresh diets were made up every 2 weeks.

Each of the treated fats was incorporated in the basal diet at a level of 10 per cent,[4] and the resulting rations were fed to mice which received the usual carcinogenic treatment. For comparison, both the control diet and a diet containing 10 per cent of untreated fat were fed. The results are given in Table IV. The groups fed oxidized and irradiated fat showed only a slightly greater incidence of tumors than those receiving untreated fat. The percentage of animals with tumors at 4½ months was 38 per cent and 40 per cent respectively, as compared with 31 per cent in the group on the untreated fat. However, when heated fat was fed, a marked increase in tumor formation resulted; 70 per cent of the animals had developed tumors at the end of 4½ months.

In an attempt to correlate chemical changes with potency for tumor formation, various fat constants were determined. A significantly higher acid number was found for the heated fat; *viz.*, 4.26 as compared with acid numbers of less than 1.0 for the other samples. This was accompanied by a reduction in iodine number in the heated fat (Table V). Taken together, the reduced iodine number and increased acid number indicated an oxidation of unsaturated fatty acid residues at the double bond. The short-chain fatty acids, released during the heating process, might therefore be regarded as particularly potent in promoting tumor formation.

The presence of peroxide, on the contrary, did not alter the tumor-promoting power of fat. Both the

TABLE III: THE EFFECT OF FEEDING VARIOUS FAT FRACTIONS ON TUMOR FORMATION WITH METHYLCHOLANTHRENE

Diet	Carcinogenic treatment	Percentage of mice with tumors at various months								Effective total
		2	2½	3	3½	4	4½	5	5½	6	
Control	0.3% MC,† 2 months	0	0	10	11	11	11	13	..	..	45 ‡
15% Fat *	0.3% MC, 2 months	13	33	46	60	60	60	..	..	..	15
15% Ethyl laurate	0.3% MC, 2 months	15	47	52	63	63	63	..	..	..	19
5% Glycerol	0.3% MC, 2 months	0	21	26	42	42	47	..	..	..	19
Unsaponifiable	0.3% MC, 2 months	0	5	15	30	35	35	..	..	..	20

* Hydrogenated vegetable oil.
† Methylcholanthrene.
‡ The control groups of 3 other series are included in these averages.

TABLE IV: THE EFFECT OF FEEDING TREATED FAT * ON TUMOR FORMATION WITH METHYLCHOLANTHRENE

Diet	Carcinogenic treatment	Percentage of mice with tumors at various months								Effective total
		2½	3	3½	4	4½	5	5½	6	
Control	0.3% MC,† 2 months	0	0	0	0	6	11	11	17	17
10% Untreated fat	0.3% MC, 2 months	0	6	31	31	31	31	31	31	16
10% Heated fat	0.3% MC, 2 months	25	25	50	60	70	70	70	70	20
10% Oxidized fat	0.3% MC, 2 months	14	19	28	28	38	38	38	38	21
10% Irradiated fat	0.3% MC, 2 months	10	30	40	40	40	40	40	40	20

* Hydrogenated vegetable oil.
† Methylcholanthrene.

TABLE V: THE CONSTANTS OF THE TREATED FATS *

	Acid number	Iodine number	Peroxide number
Untreated	0.97	64.2	4.8
Oxygenated	0.96	62.8	50-65
Irradiated	0.84	63.0	30-45
Heated	4.26	59.2	0-2

* Hydrogenated vegetable oil.

[4] At a 15 per cent level, tumor formation was so rapid with untreated fat that the additional effects of the various treatments were unrecognizable.

oxygenated and the irradiated samples, which were relatively inactive, had high peroxide numbers, whereas the heated fat, which promoted tumor formation, had a low peroxide number (Table V). No significant change was observed in any of the fat constants of the various samples when they were mixed into the basal ration and stored at room temperature for 2 weeks, a procedure similar to that used in the feeding experiments.

The effect of fat during various phases of the precancerous period.—The role of fat in tumor formation is as yet unknown. Some suggestions, however, might be revealed by data on the relative effectiveness of fat during different phases of the precancerous period. These were obtained by applying a 0.3 per cent solution of methylcholanthrene twice weekly for 2 months to mice which were receiving the high fat diet for various periods of time. Group 1 received fat throughout the precancerous period; group 2, for the first 2 months only; group 3, only after the end of the 2nd month; and group 4, beginning at 1½ months and ending 3 months after the start of the experiment. All groups received the control diet except when fat was fed, and group 5 received the control diet throughout the experiment.

Table VI: The Production of Mouse Tumors with Methylcholanthrene as Affected by Fat * Fed During Various Phases of the Precancerous Period

Diet	Carcinogenic treatment	Percentage of mice with tumors at various months							Effective total
		3	3½	4	4½	5	5½	6	
Control	0.3% MC,† 2 months	0	0	0	6	11	11	17	17
15% Fat for entire period	0.3% MC, 2 months	62	71	86	86	86	86	86	21
15% Fat for 1st 2 months	0.3% MC, 2 months	23	23	23	23	23	29	29	17
15% Fat after 2nd month	0.3% MC, 2 months	0	9	19	33	38	43	43	21
15% Fat from 1.5 to 3.0 months	0.3% MC, 2 months	33	40	53	60	60	60	60	15

* Hydrogenated vegetable oil.
† Methylcholanthrene.

The results (Table VI) indicated that the high fat diet had increased tumor production even when fed for only a part of the precancerous period. For example, when fat was fed either during the 1st 2 months or after the 1st 2 months, tumor incidence at 4½ months was 23 per cent and 33 per cent respectively, as compared with 6 per cent in the control group. However, the fat diet was more effective during the middle period than during either the earlier or the later periods; *e.g.,* tumor incidence in group 4 was 60 per cent at 4½ months. This percentage of tumor formation most nearly approached that observed when fat was fed throughout the experiment; *viz.,* 86 per cent at 4½ months.

The fact that fat was effective after the 2nd month, in other words, after the application of methylcholanthrene had ceased, suggested a physiological connection between fat and the developing tumor cell rather than a chemical connection between fat and the hydrocarbon, since methylcholanthrene disappears from the surface of mouse skin within a few days after its application is discontinued (4). The fact that the greatest effectiveness of the fat was the middle period, 1½ to 3 months after the application of hydrocarbon had been begun, suggested that the fat was involved in the final conversion of the pre-tumor cell to the tumor cell. This is the period when the tumor cell as such probably first appears.

Tumor formation in rats.—The very great effectiveness of fat in increasing tumor formation in the mouse raised the question whether the response was peculiar to this species, or common to others as well. The rat was studied in this connection, because it differs from the mouse in several important physiological particulars. The experiments were conducted as follows: Fifty-one young adult rats, ranging from 90 to 210 gm. in weight, were divided into 3 comparable groups. Tumors were produced by applying a 0.3 per cent solution of methylcholanthrene in benzene to the ears of the animals 3 times weekly for a period of 10 months. The control group was fed a low fat diet of the following composition: cooked starch, 68; casein, 18; yeast, 8; salts, 4; and cod liver oil, 2 per cent, respectively. A 2nd group received a high fat diet, which contained the same ingredients with the substitution of 30 per cent hydrogenated vegetable oil [2] for an equivalent amount of starch. A 3rd group received the low fat diet, together with applications of cottonseed oil to the ears on alternate days. These applications were included because fat applied locally to mice increases tumor formation to some extent (15, 25, 27, 29). Food and water were given ad libitum. The animals were examined for tumors at biweekly intervals, and the results expressed as percentages of the effective total (Table VII).

Tumors were found to appear at the base of the ears as small horny papillomatous warts. The first growths were observed in 9 to 10 months. They slowly gained in size, becoming round and fleshy, with only occasional bleeding and necrosis until maximum size was attained. The larger tumors were 5 to 8 cm. in diameter. Microscopic examination revealed the presence of malignant squamous epitheliomas. In each

group a few animals failed to develop tumors and instead became extremely emaciated. Such animals died in 10 to 13 months after the beginning of the experiment, without any obvious gross pathology. In general, however, growth and nutritional well-being were normal in the animals of all groups, even after small tumors had developed. An animal often survived as long as 6 months after the first appearance of a tumor.

The rates of tumor formation are shown in Table VII. There was some indication that more tumors were produced ultimately on the high fat diet than on the control diet, but the difference was by no means so marked in rats as in mice, and in the earlier months of the experiment tumors actually appeared more rapidly in control animals than in those consuming the high fat diet. The time required for the development of tumors in 50 per cent of the animals was 13 months in the control group, 12 months in the group receiving high fat, and 11 months in the group receiving local application of oil. In other words, tumors were produced most rapidly when oil was applied directly to weekly for 10 months, 5 months more were needed to reach this level of tumor incidence.

Apparently the difference is not due to a greater resistance of rat tissue to carcinogenesis *per se,* for when methylcholanthrene was injected into our rats subcutaneously in cholesterol and oil, tumors developed in 50 per cent of the animals in 4½ months (3), whereas 4 months were required for 50 per cent of our mice to develop tumors under these conditions (2). In injection experiments the hydrocarbon remains for a prolonged period in the region of carcinogenesis (5, 13, 17), whereas this does not appear to be the case when hydrocarbon is applied to the skin (4, 13). Rat tissue possesses the power to metabolize rapidly the carcinogenic hydrocarbons (5, 7, 8, 13, 22) unless they are protected somewhat, as in an oil globule or a cholesterol pellet. Since epitheliomas develop in the lower basal layer of the skin (14, 20, 21), a carcinogen applied externally must first penetrate the upper layers before reaching the proliferating cells. In relatively thick rat skin, therefore, considerable amounts of hydrocarbon could be destroyed before the region of carcinogenesis is reached. The fact that pathological changes are observed in the upper layers of skin when hydrocarbon is applied indicates that some sort of reaction between hydrocarbon and tissue takes place in the upper layers (20, 21). In very thin mouse skin on the other hand, destruction of hydrocarbon in the upper layers would be most unlikely. It is probable that local fat promotes the penetration of carcinogen in both species, but that in the mouse, penetration is adequate even in the absence of extra oil. In any event, in the mouse, fat applied locally had less effect on tumor formation than dietary fat (15, 25), whereas in the rat just the opposite was true (Table VII).

TABLE VII: TUMOR FORMATION IN RATS

Diet	Carcinogenic treatment	Percentage of rats with tumors at various months						Effective total
		10	11	12	13	14	15	
Control	0.3% MC,‡ 10 months	27	36	45	54	54	72	11
30% Fat *	0.3% MC, 10 months	15	23	54	54	77	86	13
Oil painted †	0.3% MC, 10 months	33	60	73	73	80	86	15

* Hydrogenated vegetable oil.
† Cottonseed oil.
‡ Methylcholanthrene in benzene.

the carcinogenic area. This effect was observed throughout the experiment.

The size of the tumors in the various groups paralleled the rates of tumor formation. Thus, at any one time, tumors were largest in animals receiving local applications of oil, and smallest in the control group. Hence once visible tumors had appeared, their rates of growth were essentially the same in all 3 groups.

DISCUSSION

Several explanations might be advanced for the difference between rats and mice in their sensitivities to fat as a tumor accelerator. Factors to be considered are the differences between the 2 species in the rates of tumor formation with hydrocarbons, in the rates of destruction of these agents by the body, in skin thickness, and in general well-being on diets high in fat. That skin tumors caused by hydrocarbons develop more slowly in the rat than in the mouse has been observed repeatedly (6, 11, 12, 16, 30-32). In the present experiments, when methylcholanthrene was applied to mice twice weekly for 2 months, 80 per cent of all mice on the high fat diet had tumors 1½ months later, whereas when rats on the high fat were treated 3 times

Apparently a more basic difference between the 2 species is the greater tolerance of rats for dietary fat. Rats can grow on diets containing 60 per cent of fat (9, 26), and in the present experiment 30 per cent were fed for 15 months without any untoward effect. Our mice, on the contrary, seldom survive on diets containing 25 per cent of fat; 15 per cent is ample to demonstrate an effect on tumor formation, and 10 per cent gives a measurable response. Most carcinogenic factors, such as x-rays, radium, excess ultraviolet light,

the hydrocarbons, and the azo dyes, are harmful to animals in ways other than those which lead directly to tumor formation. One effect of dietary fat in the mouse might therefore be that of a generally impaired physiological efficiency.

Other possibilities are that either the products of fat metabolism in the mouse, or the process of metabolizing large amounts of fat produce changes which favor carcinogenesis. A similar accumulation of products injected into the rat might therefore be expected similarly to increase tumor formation. Further information on the metabolic effect of fat in both species would be highly desirable. Since the tumor-promoting power of fat resides primarily in the fatty acids, a study of known degradation products might be expected to yield interesting results. In view of the observed variation in different species, any suggestion concerning the part played by fat in the etiology of human cancer should be made with extreme caution.

Summary

1. When limited amounts of 20-methylcholanthrene were applied to the skin of mice receiving a control diet, tumors developed in only 12 per cent of the animals. The addition of fat to the diet increased tumor formation to 83 per cent.

2. The tumor-promoting activity of the fat was found to reside in the fatty acid fraction. Ethyl laurate was as effective as natural glycerides; glycerol and the unsaponifiable fraction had only slight activity.

3. The action of fat was increased by heating at 300° C. for 1 hour. Rancidification with ultraviolet light, or oxygenation in the presence of copper oleate failed to alter the effectiveness of the fat for tumor formation.

4. The highest incidence of tumors appeared when fat was given throughout the experiment, but measurable increases were also observed when fat was fed either during the 1st 2 months while the carcinogen was applied, or after the 2nd month; *e.g.*, after the application of hydrocarbon had ceased. The most effective period was 1½ to 3 months after the beginning of the application of hydrocarbon.

5. Dietary fat was much less effective in promoting induced skin tumors in the rat than in the mouse. Oil applied locally increased the rate of tumor formation in rats. It is suggested that the difference between the 2 species may be due to differences in skin thickness, in the rates of destruction of the hydrocarbon, and in the ability of the 2 species to metabolize fat.

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Tumor Glycolysis

IV. The Effect of Feeding Thyroid Supplemented by Thiamin Chloride on the Growth and Glycolysis of Walker Sarcoma 319 in Rats*

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The effect of thyroid secretion on tumor growth is generally believed to be secondary to its effect on the physiological well-being; that is, when administered in quantities which increase body weight, the tumor also grows more rapidly, and when administered in quantities which cause cachexia, tumor growth is retarded (1, 4, 6, 7, 12).

Since a deficiency of vitamin B_1 is a constant finding in hyperthyroidism and can be remedied by the injection of thiamin chloride (5, 14) the effect of correcting this deficiency on tumor growth was investigated. An excess of this vitamin in the normal animal has no specific effect on the growth of tumors (9-11). Therefore, if any significant specific effect on tumor growth is observed in hyperthyroid animals in the presence of adequate thiamin chloride supply the effect could be attributed to the synergistic action of these 2 substances.

Materials and Methods

White male rats weighing 100 to 125 gm. were used. The animals in the thyroidectomized group were operated upon at least 3 weeks before the inoculation of the tumor. The success of the operation was checked by oxygen consumption studies and histologic sections of the neck region. The parathyroids were removed with the thyroids. The animals were given access to a solution containing 2.4 per cent calcium lactate in addition to their drinking water. As Richter and Eckert (15) found, the appetite of these animals for minerals was increased so that they drank enough calcium-containing water to prevent tetany and to permit a gain in weight, which normally occurs in hypothyroidism. The lactate was removed from the cage 24 hours prior to the determination of lactic acid. Lack of the parathyroid hormone and the ingestion of small amounts of calcium are considered not to affect tumor growth (7, 8, 13, 16). Those animals fed thyroid received the specified quantities (Table I), 6 days a week, of Hynson, Wescott, and Dunning dried standardized thyroid powder mixed with ground Purina chow. No other food was given the animals until this had been consumed. The rats fed thyroid plus thiamin chloride received thyroid in the same manner but were injected subcutaneously 6 days a week with the freshly made solutions of the specified quantity of Merck's betabion in 0.1 cc. physiological saline solution. The injection of thiamin chloride was controlled by injections of the same volume of physiological saline in the thyroid-fed animals. Control animals were normal rats receiving quantities of Purina chow ad libitum. Weight control animals were normal rats losing more than 5 gm. of body weight in 2 weeks because of restricted rations.

Each rat in an experimental group was inoculated subcutaneously with the tissue from the same tumor (Walker sarcoma 319). No animals from the same subgroup were inoculated successively, in order to avoid the possibility of 1 subgroup as a whole receiving less viable tissue. As soon as the tumors appeared their length and width were measured at regular intervals.

Oxygen consumption (17) determined at $26° \pm 1°$ C. after 18 to 22 hours of fasting was measured immediately before and at definite intervals after inoculation. Food consumption and weight records were kept throughout the period of tumor growth. Those weights recorded were for the early period after inoculation before the tumor attained appreciable weight.

The metabolism of the tumor was determined by a measurement of the pH fall in the living cells by means of the glass electrode, and the chemical determination of lactic acid in the excised tumor, 40 minutes after the intraperitoneal injection of 20 per cent solution of glucose. The details of the method are published elsewhere (2).

Discussion

The results summarized in Table I show that there was no significant difference in the growth rates of the tumors in the 5 groups, and that there was no consistent trend. The growth trends, in general, varied in the same direction as the body weight. Bischoff and Long have noticed that a loss of weight of 10 per cent

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is not likely to produce any great effect on tumor growth (3). These results confirm this finding. Only in Experiment 4, group A, where there was an average weight change of more than 10 per cent, was there any marked effect on tumor growth. The results provide no evidence that thyroid and thiamin acting together can produce a condition which is either favorable or unfavorable to tumor growth. A wide variation in oxygen consumption (from −20 per cent to +94 per cent of the average normal value of 197 mgm./100 gm./hour in these experiments) seemed to have no marked effect on the rate of tumor growth.

The metabolism as determined by the pH fall and lactic acid production in the tumor showed no consistent significant differences in the 5 groups. The metabolism, as measured in this experiment, as well as the growth, seems independent of (a) the oxygen consumption, (b) the thyroid hormone, (c) thiamin chloride, and (d) the combined influence of thyroid and thiamin chloride.

TABLE I: EFFECT OF FEEDING THYROID SUPPLEMENTED BY THIAMIN CHLORIDE UPON TUMOR GLYCOLYSIS IN RATS. SUMMARY OF AVERAGE VALUES

Exp. no.	Rats no.	Group no.	Tumor size mm.		Body wt. change in gm.	Daily food consumption in gm.	Oxygen consumption mgm./100 gm./hr.		Δ pH after glucose units	Lactic acid content mgm. per cent
							Before inoculation	15 days later		
1			10 days	17 days	20 days	20 days				
50 to 75 mgm.	5	A	6 x 6	13 x 12	+ 1	12.7	285	295	[— 0.08]	179
thyroid per rat	5	B	6 x 6	14 x 12	+ 7	13.2	281	305	[— 0.19]	122
	5	C	8 x 11	21 x 23	...	...	191	192	— 0.22	154
	3	D	8 x 10	19 x 19	...	...	163	157	— 0.13	151
2			14 days	19 days	19 days	19 days				
130 to 180 mgm.	2	A	15 x 12	14 x 12	— 5.5	13.4	300	356	...	...
thyroid	2	B	21 x 14	25 x 16	— 6.5	12.9	316	365	...	...
	2	B_1	18 x 13	24 x 16	— 6.0	13.1	303	347	...	...
	2	C	13 x 6	22 x 13	...	...	...	...	...	...
3			9 days	19 days	16 days	16 days				
130 mgm. thyroid	10	A	14 x 8	26 x 21	— 11.0	11.3	282	321	— 0.29	171
	10	B	15 x 10	25 x 22	— 7.0	11.4	273	322	— 0.22	154
	7	C	13 x 8	30 x 22	+ 1.0	7.0	204	185	— 0.23	147
	9	E	14 x 8	28 x 21	— 10.0	5.5	198	180	— 0.22	135
4			11 days	18 days	14 days	14 days				
180 mgm. thyroid	4	A	4 x 3	9 x 8	— 19.0	15.2	311	382	...	[250]
	5	B	11 x 7	16 x 12	— 13.0	15.4	352	366	...	[91]
	5	C	10 x 7	20 x 16	— 0.1	8.5	209	199	— 0.29	179
	2	D	8 x 13	30 x 26	+ 16.0	11.4	184	170	— 0.10	128
	5	E	6 x 5	17 x 13	— 10.0	7.7	194	205	— 0.32	188
5			11 days	18 days	14 days	14 days				
180 mgm. thyroid	4	A	14 x 9	23 x 14	+ 1.0	17.8	342	367	— 0.39	180
	4	B	11 x 7	21 x 15	— 9.0	17.1	334	369	— 0.44	185
	3	C	12 x 7	18 x 14	+ 8.0	10.3	197	199	— 0.29	179
	3	D	16 x 9	33 x 24	+ 9.0	7.9	170	169	— 0.23	154
	3	E	10 x 8	18 x 15	— 12.0	6.1	204	208	— 0.22	167

A—Thyroid-fed.
B—Thyroid-fed and 250γ thiamin chloride.
B_1—Thyroid-fed and 500γ thiamin chloride.
C—Controls.
D—Thyroidectomized.
E—Weight controls.
[ ]—One value.

SUMMARY AND CONCLUSIONS

1. An experiment was made to determine whether or not the correction of a deficiency of vitamin B_1 in hyperthyroidism would alter the effect of the hormone on tumor growth.

2. No consistent difference was observed in the growth of Walker sarcoma 319 in the 5 experimental groups; namely, (a) thyroid-fed, (b) thyroid-fed plus vitamin B_1, (c) controls, (d) thyroidectomized, (e) weight control.

3. No consistent differences in the pH drop or lactic acid production of the tumors was noted among the groups.

4. The state of hyperthyroidism in which the deficiency of vitamin B_1 was corrected had no significant effect on the growth and metabolism of Walker sarcoma 319.

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The Relation of Solar Radiation to Cancer Mortality in North America*

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Several observers (3, 6, 11) have noted that the organ incidence of cancer among peoples of European origin varies considerably with geographic location, although the total cancer death rate shows comparatively minor variations, from about 70 to 150 per 100,000 population. We find, for example, that in England stomach cancer accounts for only 22 per cent of all cancers in men, but that this rises to 42 per cent in the United States and Australia, 55 per cent in Holland and Bavaria, and 66 per cent in Czechoslovakia. Conversely the relative incidence of certain other forms of cancer decreases in the reverse order (6). One explanation of this would be that the appearance of cancer in one site confers some immunity to cancers in other sites. This conclusion, in general, is apparently supported by certain animal experiments (3, 4).

In further support of this contention some authors refer to an inverse relationship between skin cancer and the total cancer rates. Peller (11), for instance, has produced evidence that in those environments and occupations in which skin cancer is increased, other cancers are diminished; *e.g.,* in the United States Navy the skin cancer rate is 8 times that found among men of the same age range in the general population, but the total death rate from cancer of other organs is only two-fifths of the expected rate (12). Peller, therefore, suggests that we should deliberately induce cancer of the skin, which grows slowly and is easily treated, by applying light rays of suitable intensity to suitable surfaces, in order to reduce the numbers of less accessible and more malignant cancers. The total number of cases in the Navy however would seem to be too small to warrant such conclusions, and indeed more recent studies throw considerable doubt on Peller's conclusions. For instance, Conrad and Hill (2) have shown that in England and Wales, skin and total cancer mortality rates vary in the same direction. Warren and Gates (14) have also found that in a "population with cutaneous cancer there is definitely more cancer of organs exclusive of the skin than would be encountered in a similar population . . . . at large. Cancer of the skin does not protect against development of cancer elsewhere."

It is our purpose to re-examine the relationship between skin cancer and other cancers and to present evidence that the actual production of a skin cancer is not only unnecessary, but that the presence of skin cancer is really only an occasional accompaniment of a general *relative cancer immunity* in some way related to exposure to solar radiation.

Our data are derived from two sources, statistical and experimental. The latter, derived from observations extending over more than 2 years, will be published later. The former have been taken from the official figures of the United States and Canada, for the purpose of comparing the cancer and other figures from the various states and provinces. Such a comparison however involves certain difficulties.

Statistics.—Statistical accuracy is at present impossible. Although figures for all cancer cases, cured and uncured, are being collected in certain localities, we have to rely on those for fatal cases only for making comparisons between all North American states. It is probable that in some of the more scattered populations and poorer states the diagnostic facilities and the education of the people with regard to the necessity for early treatment may lag behind those of other states, thus tending to unduly high cancer death rates. In addition, a larger proportion of cancer deaths may be recorded as deaths from senility. Although these possibilities are difficult to refute, they can hardly account for the unexpectedly straight line relationships, the high coefficients of correlation and the wide range of the various graphs shown. In some graphs the figures used are the averages of the 5 years 1934-38, inclusive, kindly supplied by the Public Health authorities of the various American states and Canadian provinces. In others, the figures have been adjusted for differences of age distribution, calculated from data obtained from the United States Census figures, 1930. In most of the graphs however the gross figures have been preferred to the adjusted figures since the latter were not available for the Canadian provinces, and we wished to include the Canadian figures in order to survey as wide

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a geographical area as possible with the maximum differences in climatic conditions.

Racial differences.—Since the skin cancer rate in negroes is only about 1/7 that of white persons, it follows that a large negro population in a state must unduly lower the figures for that state. Therefore all states with a negro population of 5 per cent or more have been excluded from consideration in certain of the graphs shown. Owing to lack of information we are unable to deal with possible variations in cancer incidence due to the uneven distribution over the continent of other racial stocks.

Occupation.—Since one of the important matters in this study is the relation of skin and other cancers to solar radiation it at first seemed that we should investigate the relation of cancers to the total annual radiation in each state. Further consideration however seemed to indicate the actual number of people exposed to sunlight as a much more significant factor than the annual available radiation. A group of office clerks could not be expected to have as much skin cancer as a group of farmers, irrespective of the total solar radiation. It was not possible to obtain the proportion of each state population engaged in occupations most exposed to sunlight. Broders (1), however, has shown that the farming class provides 54 per cent of skin cancers in his experience, and farmers form by far the largest class so exposed. It was therefore decided to investigate the relation of skin and total cancer death rates in each state to the proportion of the population engaged in agriculture. The latter figures (1933) were obtained from the *National Encyclopedia,* for the United States, and from the Dominion Bureau of Statistics at Ottawa, for the Canadian provinces.

Climate, latitude, and temperature.—Any attempt to estimate the effects of these factors is unsatisfactory. We shall however in some cases refer to the mean annual temperatures of the various states as a factor in skin cancer, using figures obtained from the *National Encyclopedia* and the *Meteorological Tables* of the Canadian Department of Marine. In other cases we have employed the Solar Radiation Index calculated for each state by James Smith (13). Reference should be made to Smith's paper for the method of calculation. Consideration of the degree of exposure to wind, dusts, and other surface irritants which might act in conjunction with solar radiation has been omitted for lack of sufficient information.

It will be obvious that a correlation between cancer rates and the number of people exposed to sunlight would indicate some direct effect of the latter on cancer incidence. On the other hand a correlation between cancer and the amount of solar radiation alone would suggest rather that the effect of sunlight on cancer might be an indirect one.

The Relation of Skin Cancer Mortality to Solar Radiation

In Fig. 1 the skin cancer deaths per 100,000 population for each state in the United States and Canada are plotted against the per cent of the populations engaged in farming (farmers and farm laborers). It is at once clear that, (a) with the exception of what we shall designate the "cold state group" (the Dakotas, Minnesota, Wyoming, Alberta, Saskatchewan, Manitoba, and Quebec, all with a mean annual temperature

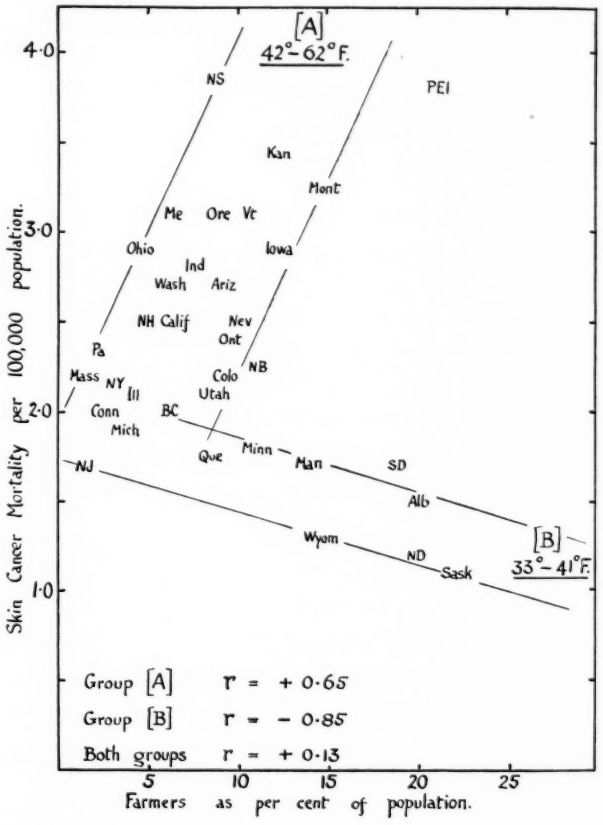

Fig. 1.—Showing the relation of skin cancer mortality rate to the proportion of farmers and farm workers in the populations of the American and Canadian states. (For calculating the values of *r*, certain states have been included in both groups; *viz.*, Mass., N. Y., Ill., Conn., Mich., N. J., B. C., and Que.)

of less than 42°F.), skin cancer deaths vary, in general, with the agricultural population. The lines of regression of these groups cut the ordinate at about 2; *i.e.*, there still remains a skin cancer rate independent of the hazards of farming and presumably due to other causes. (Vermont and Prince Edward Island also have mean annual temperatures less than 42°F., but fall in with the majority group). (b) When, however, the mean annual temperature falls to less than 42°F. (the "cold state group") we find a peculiar phenomenon; *viz.*, not only does an increase of the farming population have no effect in raising skin cancer mortality, but

it actually does the reverse and skin cancer mortality falls.

Figure 2 shows the relationship between Smith's Solar Radiation Index and skin cancer mortality rate for white populations adjusted for age. In the case of 6 states (Arizona, California, Colorado, Kansas, Nebraska, and Utah) the calculated index figures were stated by Smith to be excessive. These states are therefore omitted. Since the Canadian figures were unavailable, the general position of the "cold state group" cannot be ascertained.

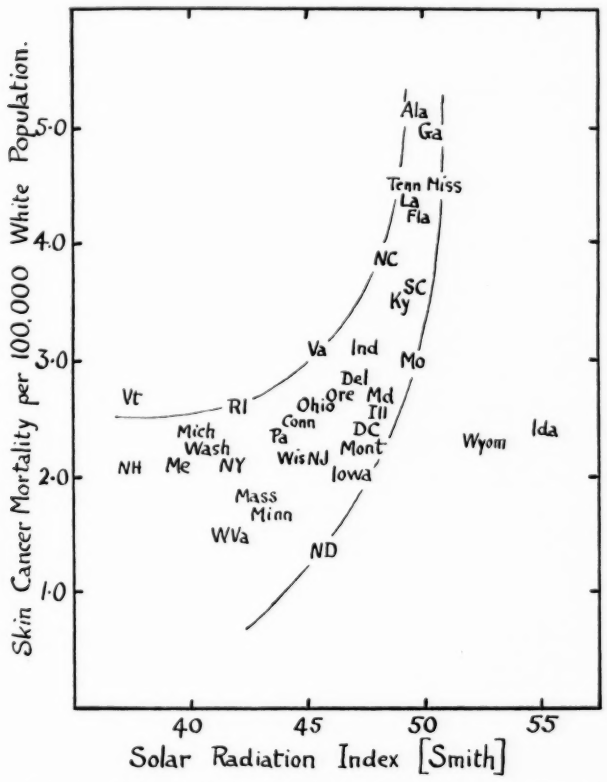

FIG. 2.—Showing the relation of skin cancer mortality rate (white population, adjusted for age) to Smith's Solar Radiation Index in the American states.

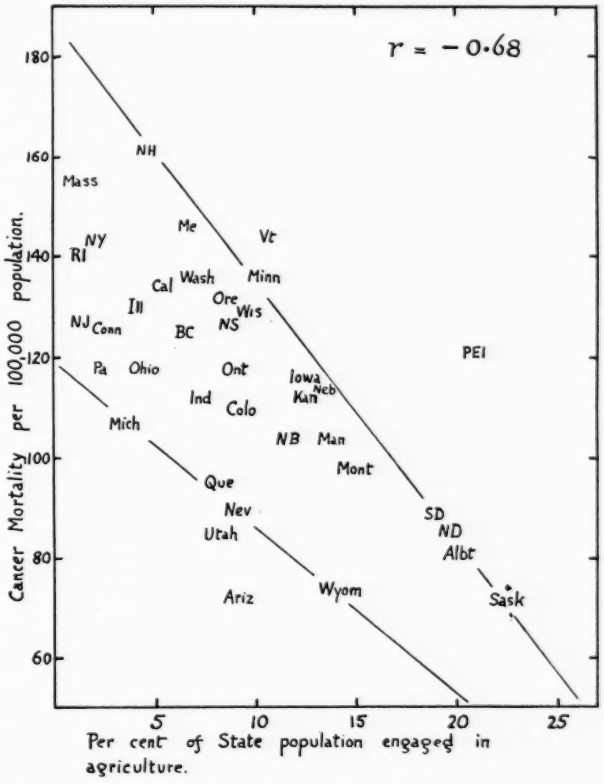

FIG. 3.—Showing the relation of total cancer mortality rate to the proportion of farmers and farm workers in the populations of the American and Canadian states.

The decline of skin cancer in the colder states might be brought about by one or both of two factors: (a) an increasing agricultural population means a diminution of the numbers engaged in those occupations which are responsible for other forms of occupational cancer, thus leading to a decrease in total skin cancers. Since we have no data on these occupations in the various states, this factor is difficult to evaluate. (b) Sunlight, of insufficient intensity to cause cancer of exposed skin, may in some other way produce a relative immunity to cancer in general, including skin cancer. This was next investigated.

The Relation of the Total Cancer Mortality to Solar Radiation

Figure 3 shows the relation of the total cancer mortality per 100,000 population to the farming population. Fig. 4 shows the same relationship for cancers per 100,000 people over the age of 45. Fig. 5 shows the relation of total cancer (white population) to the Solar Radiation Index of Smith (13). These results have been evaluated statistically and the coefficients of correlation (r) recorded on each graph. According to Snedecor's *Statistical Methods* (1937) these coefficients are highly significant. It is therefore evident from these graphs that the general cancer rate declines with increasing solar radiation and with increasing numbers of people exposed thereto.

It now seems clear that we are dealing with the operation of two mutually opposing forces; *i.e.*, that solar radiation or something closely associated therewith, has two separate effects, (a) it produces some sort of relative immunity to cancer in general and, in those localities where the mean temperature is less than about 42°F., even to skin cancer, but, (b) at mean temperatures above 42°F., solar radiation produces more cancer on those parts of the skin exposed thereto, in spite of a generally raised immunity.

These generalizations help us to understand why different observers have failed to show any consistent correlation between skin cancer and total cancer; *e.g.*, Peller's population, living in warmer climates, shows an inverse relationship, whereas the relationship is a direct one in the colder and less sunny climates of England and Wales (2).

Data From Other Countries

For comparison with the North American figures it is of interest to cite those from various other countries at different latitudes. However, many difficulties of interpretation and other indeterminate factors arise. Thus it is probable that the facilities for the diagnosis of cancer are not as adequate in many of the tropical countries as in those of more temperate climates, and therefore their statistics are less reliable. On the other hand in cities diagnosis is likely to be more accurate. It is therefore more significant to compare figures for cities arranged in groups according to latitude. Table I, from Hoffman (5), shows the progressive decline in total cancer mortality with approach to the equator. Also, the total cancer rates in southern Europe (Italy, Spain, Portugal, and southern France) are much lower than those in northern Europe, even when corrections are made for differences of population ages (5, 7).

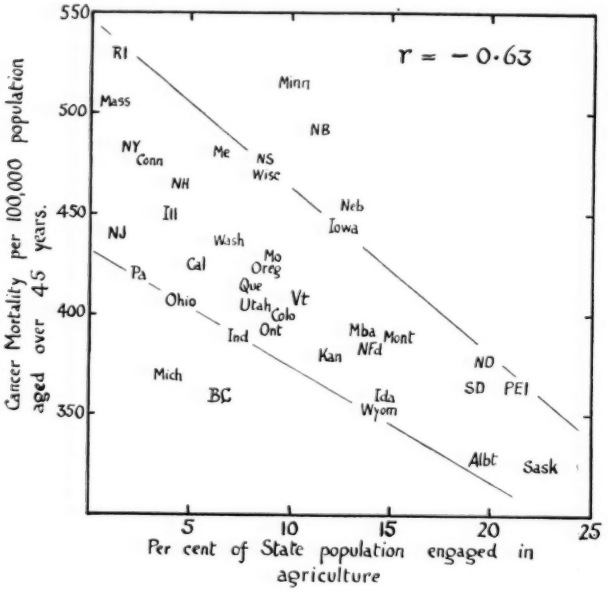

FIG. 4.—Showing the relation of total cancer mortality in that part of the population aged 45 years or more, to the proportion of farm workers in American and Canadian states.

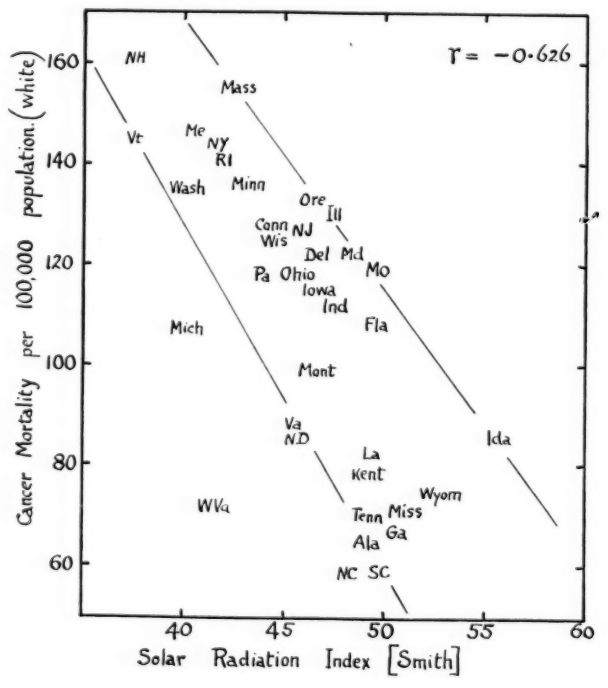

FIG. 5.—Showing the relation of total cancer mortality rates to Smith's Solar Radiation Index in the American states, (white population only).

TABLE I: MORTALITY FROM CANCER IN CITIES ACCORDING TO LATITUDE, 1908-12 *

Number of cities	Degrees of latitude	Deaths from cancer	Rate per 100,000 population
35	60 N—50 N	119,374	105.7
48	50 N—40 N	121,216	92.4
24	40 N—30 N	37,451	78.1
7	30 N—10 N	5,696	42.3
4	10 N—10 S	1,056	40.9
7	10 S —30 S	3,040	37.7
5	30 S —40 S	11,048	89.8

* Modified from Hoffman (5).

The Nature of the Relation of Solar Radiation to Cancer Mortality

Assuming the truth of our thesis concerning an inverse relationship between total cancer and exposure to solar radiation, we must next consider the nature of that relationship. There are two possibilities:

(a) That the relative immunity from cancer in farming states is an indirect effect, possibly brought about by some beneficent effect of solar radiation on the larger amounts of foodstuffs produced in these states. Although with modern transport these foodstuffs are probably fairly evenly distributed and consumed over the continent, it is also probable that the more expensive green vegetables and raw foods would be less available to the low wage masses in the manufacturing states and cities than to those in the farming states which produced the food. In that case, all cities, irrespective of latitude, would have nearly equally high cancer rates. As Table I shows, however, cancer in cities also falls with approach to the equator and increasing solar exposure.

(b) That relative immunity to cancer is a direct effect of sunlight. The only direct evidence of which we are aware that bears on this question is certain experimental work on animals which, however, as always, must be applied with caution and reserve to human problems. Pierce, Van Allen, and Brown (9, 10) have found in a series of experiments extending over 4 years, that constant light (Mazda and mercury arc lamps) lowers the malignancy (*i.e.*, incidence, mortality, and number of metastases) of transplanted

cancers in rabbits. More recently Morton *et al.* (8) found that, of 2 groups of mice painted with benzpyrene for 17 weeks, the group exposed to artificial daylight for 12 hours daily had fewer tumors (papillomas and skin cancers) than had the group kept in darkness. The appearance of the tumors was also delayed. Our own incomplete experiments with cancer-strain mice over a period of more than 2 years seem to point to a similar conclusion.

Conclusions

1. The apparent discrepancy between the views of those who claim that skin cancer and the general cancer rates vary inversely when different localities are compared, and of those who claim a direct relationship between these two groups of cancer, is shown to be a matter of climate. In hot climates the relation is an inverse one, in cold climates a direct one.

2. The total cancer mortalities of the various American states and Canadian provinces are shown to fall with increasing solar radiation and with the number of people exposed thereto, and are independent of the production of skin cancer. The fall of skin cancer with increased exposure in cool climates is merely one example of this general rule. In warmer climates, however, skin cancer may indeed rise in spite of the relatively increased general immunity. In other words the production of skin cancer is not necessary for the appearance of general cancer immunity, as claimed by some observers, but is merely an occasional accompaniment.

3. It is suggested that we may be able to reduce our cancer deaths by inducing a partial or complete immunity by exposure of suitable skin areas to sunlight or the proper artificial light rays of intensity and duration insufficient to produce an actual skin cancer. A closer study of the action of solar radiation on the body might well reveal the nature of cancer immunity.

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The Effects of Spontaneous and Transplanted Rat and Mouse Tumors on the Red and White Cells in Circulating Blood and Bone Marrow*

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The results of experiments reported by a number of investigators have led to the conclusion that the reactions of the host against tumor grafts are due to an immunity which develops against the transplanted tissues, and that this immunity is specific for the tumor. Thus DaFano (8) noticed in mice that after the transplantation of a piece of carcinoma of the mammary gland, lymphocytes and monocytes accumulated around the graft and became more numerous in the peripheral blood; he attributed to these cells the function of initiating an immunity against the tumor. Similarly, Murphy and Morton (22, 23) gave much attention to the behavior of the lymphocytes in the reactions against transplanted tumors. According to these investigators, an increase in the number of circulating lymphocytes is due to the development of an immunity against the tumor graft. Baeslack (1) held that a lack of immunity is manifested by an increase in the number of circulating polymorphonuclear leucocytes; he noticed that if a transplanted tumor grows continuously, the number of polymorphonuclear leucocytes in the circulation of the host increases. More recently, Lewis (10) has likewise observed that as the transplanted tumor increases in size, the number of polymorphonuclear leucocytes in the peripheral circulation increases, and that the white blood cell count is highest just previous to the death of the host.

However, the fact that the typical reaction occurring after homoiotransplantation of tumor is similar to that seen after homoiotransplantation of normal tissues seems to support the interpretation of Loeb (12-21) that the organismal differentials of normal tissues and of tumor tissues are similar or identical, and that they are responsible also in transplanted tumors, at least partly, for these changes. Furthermore, as Loeb (12-21) has pointed out, this conclusion is also sustained by the fact that normal homoiogenously transplanted tissues are able to elicit an immunity against homoiotransplanted tumors, as observed by Schöne (25) and Ehrlich (9).

Recently we (7) have been able to show that the organismal differentials of transplanted normal tissues exert a distant effect in addition to the local effect, in that after homoiotransplantation they cause a typical increase in circulating lymphocytes, whereas, after heterotransplantation of various organs an increase in polymorphonuclear leucocytes is observed in the circulating blood. It will be of great interest to compare the effects on the distribution of blood cells after transplantation of normal tissues and of tumors.

Such investigations may be expected to decide whether the blood cell changes following tumor transplantation are due to conditions applying specifically to tumors or to organismal differentials present in tumors and similar to those in normal tissues. Accordingly, in a series of experiments the changes in the counts of white and red cells in the circulating blood following homoio- and heterotransplantation of various tumors in mice, rats, and guinea pigs were studied. In addition, counts were made in animals with spontaneous tumors, with or without autotransplantation of tumor pieces, in order to determine whether autogenous tumor tissues behave like autotransplanted normal tissues during their transformation into cancers.

The effect of homoiotransplantation of tumors on the blood counts.—Altogether, 195 homoiotransplantations of tumors were carried out, as shown in Table I. Of these, 126 were in rats and 69 in mice. In 40 rats, Flexner-Jobling carcinoma was used; in 55 additional rats, the R39 sarcoma; and in 19 rats, the Walker carcinoma. All but 18 (14.3 per cent) of the rats showed early changes in the peripheral blood which were similar to those observed by us after homoiotransplantation of normal tissues; those which did not show this typical reaction are also included in the averages computed in Table I. In those reacting positively there was an increase in lymphocytes exceeding the normal number by 10 per cent or more;

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the average maximum increase in all the rats receiving homoiotransplants of tumor was 13.7 per cent. This increase occurred as early as on the 4th or 5th day and persisted for 8 to 10 days thereafter. A maximum was reached in most instances between the 5th and 8th day, although in some rats it occurred as early as after 4 days, and in others as late as the 12th day; on the average, it was 5.7 days after transplantation. In 5 cases the lymphocytic increase was in excess of 10 per cent until the 12th day, when it began to return to normal; whereas in all other positive cases the counts returned to the normal level by the 15th day. This reaction occurred whether or not the transplantation resulted in an actively growing tumor; 16 of the 106 rats in which a positive reaction was observed did not have actively growing tumors. However, in those cases in which the reaction was not typical the tumor failed to grow.

In another group of experiments a benign rat adenofibroma was homoiotransplanted and blood counts were made. In all 12 experiments of this kind there was an increase in lymphocytes, but there was a great variation as to the time at which the maximum count was obtained; it varied from 7 to 15 days, and, on the whole, was not as high as that observed after homoiotransplantation of more actively growing tumors in the rat. In the latter experiments, the increases in positively reacting cases ranged from 10 to 38 per cent, with an average of 13.7, whereas in the experiments with the benign tumor 20 per cent was the greatest increase recorded, with an average maximum rise of 11.3.

Similarly, experiments were carried out in mice in which tumors were transplanted within the same strain, as well as from one strain to another. There were 47 transplantations of the former and 22 of the latter type. The kind of reaction did not differ in these 2 series, except that after transplantation within the same strain the lymphocytes in the peripheral blood did not increase as rapidly as after transplantation of the tumor into a different strain. Thus, as can be seen in Table I, the average day of maximum increase was 7.3 when transplantation was carried out within the same strain, and 6.0 when the transfer of tumor was from 1 strain to another. There was also a tendency for the lymphocytic count to remain elevated for a longer time when transplantations were within the same strain; thus in the experiments of this type the counts frequently exceeded 10 per cent even as late as 15 days after transplantation. But in these cases, as well, the counts returned to the normal level by the 20th day. When the tumor transplantations were carried out from a mouse of one strain to mice of another strain, the tendency was for the count to rise more rapidly, reaching a maximum in most cases between the 4th and 6th day, and then to decline to the normal state more rapidly; by the 15th day, all of these mice showed normal lymphocytic counts. In these experiments the reactions occurred also when the tumor failed to grow, as was usually the case when a transplantation was made from one strain to another.

We next attempted to determine wherein, in animals with actively growing tumors, the counts taken at later periods after transplantation differed from

TABLE I: EFFECTS UPON LYMPHOCYTES FOLLOWING HOMOIOTRANSPLANTATION OF TUMORS INTO 126 RATS AND 69 MICE *

Strain of tumor	Number of animals	Maximum lymphocytic increase: Per cent, Average		Per cent, Maximum	Day of occurrence, Average		Day of occurrence, Maximum
1. In rats of a different strain							
Flexner-Jobling carcinoma	40	13.4	13.7	38	6.5	5.9	12
R39 sarcoma	55	13.5		21	6.4		10
Walker carcinoma	19	14.8		25	6.2		10
Wolfe adenofibroma	12	11.3		20	7.9		11
Number of animals—126.							
2. In mice of same strain							
Strain D carcinoma	5	27.2	17.2	38	6.8	7.3	10
Strain C3H carcinoma	20	19.2		29	7.7		10
Strain A carcinoma	14	13.1		19	6.8		8
Strain D sarcoma	8	12.9		25	7.5		12
Number of animals—47.							
3. In mice of a different strain							
Strain A carcinoma to strain D mice	7	20.3	17.3	26	6.0	6.0	10
Strain C3H carcinoma to strain AKA mice	10	12.4		22	5.6		10
Strain C3H carcinoma to strain CBA mice	5	22.8		30	6.6		8
Number of animals—22.							
Total number of animals in parts 1, 2, and 3—195.							

* The strain D sarcoma was originally produced by long-continued injections of a buffered solution of HCl by Suntzeff, Babcock, and Loeb (26). We are indebted to Dr. Leo Loeb for the mouse tumors, to Dr. G. A. Wolfe for the benign adenofibroma, and to Dr. W. H. Woglom for the 3 malignant rat tumors used in these experiments.

those in which the tumor did not proliferate to any noticeable extent. Since Baeslack (1) and also Lewis (10) have shown that as the tumor increases in size, the number of polymorphonuclear leucocytes increases in the circulating blood, it was decided to extend the period during which the counts were taken from 40 to 60 days after transplantation of the tumor. It was observed in both the experiments with rats and in those with mice in which transplantations were carried out with the same strains, that some time within 30 to 50 days following transplantation of the tumor the polymorphonuclear leucocytes began to increase in number, and continued to remain elevated until the animal died. It was furthermore noted that this increase did not take place unless the tumor had reached a certain critical size; this point coincided with the time when the animal was becoming debilitated as a result shown in Tables II and III, where it can be seen that while the red cell count remained remarkably constant in animals in which the tumor transplant did not take, there was a marked drop in the number of erythrocytes in animals bearing large, progressively growing tumors. The average normal red cell count in mice was about 10 million per cu. mm. of blood, whereas 45 days after transplantation of the tumor, it had fallen to 3.51 million per cu. mm., on the average; in 4 cases it was as low as 2.5 million per cu. mm., or less. The counts in rats showed a similar decline from approximately 9 million per cu. mm., to an average of 3.41 million red cells per cu. mm., but these occurred only 50 to 60 days after transplantation; in consequence of the larger size of rats, the tumor required a longer time to reach a size causing marked anemia.

TABLE II: THE EFFECT OF ACTIVELY GROWING TUMORS ON THE PERIPHERAL RED AND WHITE BLOOD CELL COUNTS

Tumor transplanted	Number of animals	Counts at time of transplantation				Counts 40 to 60 days after transplantation			
		Rbc.* per cu. mm.		Wbc. per cu. mm.		Rbc. per cu. mm.		Wbc. per cu. mm.	
		Average	Maximum	Average	Maximum	Average	Maximum	Average	Maximum
In rats without tumors									
R39 sarcoma	6	9.37	9.63	9,300	10,250	9.13	9.45	9,750	10,400
Walker carcinoma	8	9.07	9.30	10,200	10,700	9.08	9.21	9,900	10,350
In rats with tumors									
R39 sarcoma	9	9.28	9.45	10,100	10,650	3.12	3.45	24,300	29,050
Walker carcinoma	7	9.37	9.58	10,250	10,800	3.78	4.13	18,750	21,000
In mice without tumors									
Strain D carcinoma	5	10.47	10.62	10,350	11,200	10.15	10.51	9,950	10,200
Strain A carcinoma	5	10.24	10.51	10,200	11,050	10.32	10.61	10,300	11,150
Strain C3H carcinoma	5	10.19	10.39	10,250	10,900	10.16	10.43	10,150	10,300
In mice with tumors									
Strain D carcinoma	5	9.97	10.30	10,100	10,450	3.34	4.73	19,300	25,000
Strain A carcinoma	10	10.33	10.67	10,350	11,200	3.62	4.97	31,750	40,050
Strain C3H carcinoma	5	10.03	10.71	10,050	11,200	3.46	4.85	24,650	31,150

* Rbc. represents the number of millions of erythrocytes per cubic millimeter of blood.

of the large size of the tumor. The extent of the increasing loss of strength could be judged by the degree of anemia.

Changes in the red blood cell counts were specially studied in 15 rats implanted with the R39 sarcoma and in 15 rats implanted with the Walker carcinoma, as well as in 35 mice receiving implants of a transplantable mammary carcinoma. In 6 of the rats with the R39 tumor and in 8 with the Walker carcinoma, the tumor failed to grow and there were no significant changes in the erythrocyte count of these animals throughout the experiment. In all other animals a marked anemia developed when the tumor had grown to a large size; at this time there was also a considerable increase in the number of polymorphonuclear leucocytes. Similarly, the red blood cell counts were compared in 15 mice in which the tumor transplants failed to grow, with those of 20 mice with actively growing tumors. The results of these experiments are

The changes observed in the red cells circulating in the blood vessels, as well as those situated in the bone marrow, agreed with what is usually seen in a secondary anemia. The red cells in the blood varied greatly in size and shape. There was an increased number of red cells with basophilic stippling, as well as of reticulocytes, the number of the latter amounting from 5 to 10 per cent as compared with the normal of less than 1 per cent. Furthermore, normoblasts were seen in the peripheral blood of animals with large tumors, whereas they were absent in the blood of rats and mice in which the tumor transplant failed to grow. In the bone marrow there was an increased number of immature red cells, particularly normoblasts and red cells with basophilic stippling.

The relationship of the size of a tumor to the blood changes can be seen in comparing the data shown in Table III with that of Table IV: in these tables the counts taken in individual animals are recorded. In

Table III it can be seen that with large tumors there is a marked anemia, in which the erythrocyte count falls to about 3 million cells per cu. mm.; in 4 mice, the red blood cell counts were 2.5 million or less. A leucocytosis is also present at this time; in the animals recorded in Table III the increase ranged between about 5,000 and 31,000 white blood cells per cu. mm.

Table III: The Effect of Actively Growing Tumors on the Peripheral Red and White Blood Cell Counts

Tumor transplanted	Number of animal	Counts at time of transplantation		Counts 40 to 60 days after transplantation	
		Rbc.	Wbc.	Rbc.	Wbc.
In rats					
R39 sarcoma	1	9.32	9,750	3.45	14,050
	2	9.17	9,900	3.20	18,100
	3	9.41	10,200	3.01	25,650
	4	9.24	10,050	3.07	29,070
	5	9.33	10,250	3.16	28,400
	6	9.08	10,650	3.14	27,000
	7	9.45	9,800	3.02	24,350
	8	9.30	10,350	3.06	25,500
	9	9.27	9,950	3.09	26,600
Walker carcinoma..	1	9.58	9,600	4.02	20,350
	2	9.21	10,650	3.94	14,950
	3	9.13	10,300	3.32	17,050
	4	9.47	10,800	3.59	20,600
	5	9.53	10,050	3.61	19,150
	6	9.45	10,150	4.13	21,000
	7	9.37	10,200	3.85	18,200
In mice					
Strain D carcinoma.	1	9.73	10,300	3.21	17,300
	2	9.89	9,850	2.96	25,000
	3	10.30	9,700	4.73	18,200
	4	10.25	10,450	2.50	15,600
	5	9.68	10,200	3.97	20,400
Strain A carcinoma.	1	10.67	10,650	3.42	34,200
	2	10.31	9,900	3.12	25,500
	3	9.83	9,450	3.43	31,150
	4	9.91	10,500	4.14	40,050
	5	10.40	11,200	4.08	38,700
	6	10.52	10,450	3.67	33,450
	7	10.42	10,150	4.97	35,300
	8	9.70	10,550	4.01	22,800
	9	10.33	10,300	3.57	27,250
	10	10.21	10,450	2.39	29,100
Strain C3H carcinoma	1	10.71	10,050	3.45	21,150
	2	9.98	11,200	2.44	31,150
	3	10.25	9,450	4.85	18,900
	4	9.92	10,650	2.42	27,300
	5	9.89	9,100	4.14	24,850

Animals which are bearers of small tumors do not show significant changes in their blood counts, regardless of whether the tumor subsequently retrogresses completely or continues to grow to a large size. The greatest decrease in erythrocyte count in animals with small tumors (Table IV) was 2.39 million, and the red cell count in this mouse was 7.95 million. However, most of the animals showed changes of less than 1 million red cells; in fact, in 10 of the 30 animals with small tumors there was an increase in the red blood cell count during the early period of tumor growth. Similarly, the rise in the white blood cell counts ranged between 1,000 and 3,800 leucocytes per cu. mm.; 11 of these 30 animals showed a decrease rather than an increase in leucocyte counts. It appears, therefore, that the counts in animals recorded in Table IV represent a normal variation rather than an effect which can be attributed to the presence of a small tumor.

Table IV: The Effect of Actively Growing Tumors on the Peripheral Red and White Blood Cell Counts

Tumor transplanted	Number of animal	Counts at time of transplantation		Counts 15 to 20 days after transplantation	
		Rbc.	Wbc.	Rbc.	Wbc.
In rats					
Flexner-Jobling carcinoma	1	9.95	11,100	10.01	10,200
	2	10.05	9,650	9.97	8,700
	3	8.96	8,700	9.85	10,250
	4	10.21	10,350	9.46	9,600
	5	10.56	9,900	10.22	10,600
Walker carcinoma..	1	9.72	8,550	10.10	11,600
	2	10.24	8,200	10.41	8,550
	3	10.02	9,550	9.58	10,300
	4	9.18	10,050	8.72	8,950
	5	10.34	10,350	9.60	13,150
R39 sarcoma *	1	10.08	10,150	10.82	9,450
	2	10.16	8,950	10.38	9,200
	3	11.48	8,300	11.28	10,100
	4	9.50	10,450	8.74	11,600
	5	9.74	9,400	9.61	12,250
In mice					
Strain C3H * carcinoma	1	10.69	7,900	9.31	8,750
	2	10.32	8,650	9.54	9,150
	3	11.06	9,250	10.91	8,600
	4	10.97	10,050	10.52	10,400
	5	11.32	8,350	11.76	9,400
Strain D carcinoma.	1	11.13	9,200	8.74	8,700
	2	10.07	10,150	9.01	10,300
	3	9.20	8,750	10.13	9,550
	4	9.84	9,450	7.95	10,400
	5	10.30	10,200	10.52	11,100
Strain A carcinoma.	1	10.02	7,500	9.97	8,950
	2	11.73	8,750	10.71	9,300
	3	10.65	8,200	10.52	8,450
	4	11.40	9,650	10.95	10,200
	5	9.95	10,050	10.04	9,950

* In these 2 groups the tumors subsequently retrogressed completely, but there were small tumors present at the time the counts in the last 2 columns were taken. In the other 4 groups the tumors grew progressively larger and eventually showed the typical anemia and leucocytosis.

The effect of heterotransplantation of tumors on the polymorphonuclear leucocytes in the peripheral blood.

—A total of 64 heterotransplantations of tumors was carried out, as shown in Table V. The typical reaction to these tumors was similar to that which we have observed following the heterotransplantation of normal tissues. There was an increase in polymorphonuclear leucocytes in the peripheral blood, which began usually between the 2nd and 4th day after the transplantation, and reached a maximum between the 5th and 9th day. The average increase in polymorphonuclear leucocytes in all the animals in which tumors were heterotransplanted was 16.4 per cent, with a range of increase varying in individual animals between 8 and 42 per cent; the average day of maximum increase was 5.7. Nine additional experiments, 12.3 per cent, not included in Table V, failed to show a typical polymorphonuclear leucocyte increase.

It may further be concluded that the reaction following the heterotransplantation of tumors is similar to that following the same kind of transplantation of normal tissues. There is an increase in the number of polymorphonuclear leucocytes, which, when tumors are the heterotransplanted tissues, tends to reach a maximum a day or two later than when normal tissues are so transplanted; this is followed in both instances by an increase in lymphocytes, which reaches a maximum 16 to 18 days after transplantation.

The effect of growth of spontaneous tumors on the polymorphonuclear leucocytes, red blood cells, and bone marrow.—The changes in total red and white blood cell counts and the relative proportion of lymphocytes and polymorphonuclear leucocytes in the peripheral circulation were studied in 12 strain D

Table V: The Effect of Heterotransplantation of Tumors on the Polymorphonuclear Leucocyte Count

Strain of tumor	Number of animals	Maximum polymorphonuclear leucocyte increase: Per cent — Average	Per cent — Maximum	Day of occurrence — Average	Day of occurrence — Maximum
1. Rat tumors to guinea pigs					
Flexner-Jobling carcinoma	6	17.7	23	6.0	8
R39 sarcoma	6	25.6	42	6.2	8
Wolfe adenofibroma	5	10.3	13	9.0	12
2. Rat tumor to mice					
R39 sarcoma to strain A mice	5	12.5	16	6.7	9
		16.4		5.7	
3. Mouse tumors to rats					
Strain D sarcoma	11	17.0	27	5.9	8
Strain A carcinoma	11	16.4	26	4.9	8
4. Mouse tumor to guinea pigs					
Strain D sarcoma	20	15.3	32	4.7	8

Number of animals—64.

By the 12th day the count in most cases had fallen to normal. One to 2 days later a rise in lymphocytes set in. This usually reached a maximum between the 16th and 18th day after transplantation, after which the counts returned to normal. The average secondary lymphocytic increase was 16 per cent, with a range of variation in individual experiments between 7 and 32 per cent. All but 3, 4.7 per cent, of the cases which manifested the early increase of polymorphonuclear leucocytes showed this secondary reaction.

Five additional mice received heterotransplantations of either thyroid or liver. In these, as well as in the 5 mice which received the R39 rat sarcoma, total erythrocyte counts were taken before transplantation and at 2-day intervals during the time at which the polymorphonuclear leucocyte increase was noted. These counts showed no significant deviation from the normal. It may therefore be concluded that the increase in polymorphonuclear leucocytes following heterotransplantation is not associated with an anemia such as was observed when the leucocytic increase occurred in animals bearing spontaneous or homoiogenous tumors.

mice with spontaneous mammary carcinoma. In 5 of these cases counts were begun when there was a small palpable tumor, while in the other 7 mice the tumors were somewhat larger. During the early period of growth, the total white blood cell count was, on the average, 11,400 cells per cu. mm., with variations in individual mice ranging between 9,800 and 16,100 per cu. mm., while the red count was, on the average, 11 million cells per cu. mm., with a range of variation between 10 million and 14.16 million. There were no significant changes in the relative numbers of lymphocytes and polymorphonuclear leucocytes until the tumors became moderately large; from that time on there was a gradual increase in the total white cell count and in the relative number of polymorphonuclear leucocytes. Concomitantly, a decrease in the total number of red cells and an accompanying increase in reticulocytes occurred; normoblasts were present also in the peripheral blood when the later stages of the anemic condition were reached. When the tumors became large, the average erythrocyte count fell to 4.12 million cells per cu. mm., with a range of variation between 1.3 and 6.74 million cells per cu.

mm. The average would probably have been even lower had we permitted some of the tumors to grow further. The total white blood cell count was, on the average, 25,200 per cu. mm., and ranged between 15,350 and 45,000 cells per cu. mm.; the percentage of polymorphonuclear leucocytes in the differential count varied between 42 and 80 per cent, as compared with the normal variation between 20 and 35 per cent. Thus there was both a relative and an absolute increase in polymorphonuclear leucocytes. The normal figures were obtained from counts in 6 strain D mice of the same ages as those with spontaneous tumors.

The results of counts in mice taken at periods late in the development of mammary gland carcinoma are similar to those observed during a comparable period in the experiments with actively growing, homoiotransplanted tumors, in that there was an increase in total white count and in polymorphonuclear leucocytes, and a decrease in red blood cells, associated with an increase in reticulocytes and the presence of normoblasts. Furthermore, the bone marrow changes were similar in both types of experiments. In mice with spontaneous tumors there was likewise an increase in normoblasts and basophilic erythrocytes, as well as an increase in the number of neutrophilic granulocytes similar to that in mice in which homoiotransplanted tumors had attained a large size. There was, however, a difference between these 2 groups inasmuch as the mice receiving homoiotransplants showed an early increase in lymphocytes, whereas this reaction was absent in mice with spontaneous tumors.

The effect of autotransplantation of tumors on the leucocytes of the blood.—In 4 strain D mice, with small spontaneous tumors, autotransplantations were carried out in order to compare blood changes in this type of transplantation with those following autotransplantations of normal tissues. As in the case of normal autografts, there was here a slight rise in lymphocytes, presumably due to the operative interference, but the count returned to normal in all cases within 4 days after the operation. Following this period the differential count did not vary significantly until approximately 40 days after transplantation, when the tumor had become moderately large; then the same change in red and white cell counts occurred which we have observed in animals bearing spontaneous or large homoiografted tumors.

The effect of various types of double transplantations on the leucocytes of the blood.—We have previously reported that following a 2nd homoiotransplantation of normal tissue, made 12 or 20 days after the 1st homoiotransplantation, there is a more rapid rise in the number of lymphocytes, but this rise is not as great as that following the 1st transplantation. Similar experiments, which we have divided into 3 groups, have been carried out with transplanted tumors: (a) a 1st transplant of tumor followed by a 2nd transplant of normal tissue; (b) a 1st transplant of tumor followed by a 2nd transplant of the same tumor; (c) a 1st transplant of normal tissue followed by a 2nd transplant of tumor.

Group 1.—Four rats received homoiotransplants of the R39 sarcoma and 4 other rats received homoiotransplants of the Flexner-Jobling carcinoma. All 8 rats showed the typical early rise in lymphocytes. Twenty days later, when the tumors were not yet large enough to cause an increase in polymorphonuclear leucocytes, these rats received homoiotransplants of thyroid gland; at this time the differential lymphocytic count had again returned to normal. Within 2 to 4 days following the 2nd grafting there was an average increase in lymphocytes of 14 per cent, with individual counts varying between 8 and 21 per cent. In 1 rat in each of the 2 groups the increase was somewhat greater on the 6th than on the 4th day, but then the count returned to normal. In the other 6 rats the return to normal followed after the 4th day. Thus there was the same kind of effect exerted by the 1st piece of homoiotransplanted tumor on the 2nd graft of normal tissue as that which was formerly observed when also the 1st graft consisted of normal tissue.

In 5 other experiments with strain C3H mice a 2nd homoiotransplantation of normal tissue, after a tumor had first been transplanted, was made when the tumor was large enough to cause a polymorphonuclear leucocytic reaction. The total white cell count remained elevated to the same degree in 2 of the mice and rose even to a higher level in the other 3 animals for 4 days following the 2nd transplantation of normal tissue. In all 5 mice it dropped slightly during the succeeding 4 days and then rose again. Differential counts showed that during the 4 days following the 2nd transplantation of normal tissues there was an absolute increase in lymphocytes.

Group 2.—In a series of 10 experiments in mice of the C3H strain a primary homoiotransplantation of tumor was made, followed 40 days later by a 2nd homoiotransplantation of the same tumor. At the end of 40 days, 6 of the 10 mice showed the polymorphonuclear leucocytic increase characteristic of animals bearing large tumors. When these 6 mice received a 2nd homoiotransplant of the same tumor, the polymorphonuclear leucocyte count showed an average decrease of 12 per cent, while the lymphocytes increased correspondingly; this occurred between the 2nd and 4th day following the 2nd grafting of tumor. Subsequently the count of polymorphonuclear leucocytes began to rise.

In the other 4 mice the tumors were not large enough at the end of 40 days to cause an increase in polymorphonuclear leucocytes; in these, after a 2nd grafting of tumor, there was an average increase in

lymphocytes of 14 per cent between the 2nd and 4th day, after which the counts returned to normal. From the 15th day on, the polymorphonuclear leucocytes rose; by that time the tumors had reached a large size.

In these experiments, then, the response to a 2nd transplantation of a tumor was similar to that following the 2nd transplantation of normal tissue, although in 6 of the mice the response was modified when the tumors became large enough to cause an increase in polymorphonuclear leucocytes. This modified effect consisted in a temporary decrease in polymorphonuclear leucocytes and a corresponding increase in lymphocytes.

Group 3.—In 5 experiments in C3H mice, homoiotransplantation of normal tissue was followed 12 days later by the transplantation of a piece of tumor. Again there was, following the tumor transplantation, an acceleration of the lymphocytic rise similar to that which occurs when both primary and secondary transplants consist of normal tissues. In 4 of these mice the reaction to the tumor transplantation was apparent as early as on the 2nd day, and reached a maximum on the 5th day; in the 5th mouse the maximum reaction was reached on the 2nd day and the increase in lymphocytes was greater than that which occurred after a 1st transplantation of normal tissue. In the other 4 mice the lymphocytic increase following the tumor transplantation was not as great as that which has occurred after the 1st transplantation of normal tissue.

The specificity of this reaction is indicated by an experiment in which 5 C3H mice received a heterotransplantation of normal rat tissue 12 days before they had received a homoiotransplantation of tumor. In this experiment the heterotransplantation failed to cause an acceleration of lymphocytic increase.

Discussion

Transplantation of normal tissues has shown that the relationship between the individual and species differentials of host and transplant determines the reaction of the host cells—lymphocytes, polymorphonuclear leucocytes, connective tissue cells, and capillary endothelium—towards the transplant, and, consequently, the fate of the latter, as shown by Loeb (12-21). In a similar way the fate of tumor transplants is largely determined by the genetic relationship between tumor and host, as first observed by Loeb in comparing the results of auto- and homoiotransplantation of tumors. Loeb concluded that it is the relationship between the organismal differentials of host and transplant on which the outcome of tumor as well as of normal tissue transplantations largely depends. In some essential respects the fate of transplanted tumors is therefore determined by factors similar to those which determine the fate of normal tissues, although there are some minor differences.

Bashford and Russell (2), Tyzzer (28), and others, believed that it is the immunity or resistance which tumors evoke in the host which determines the fate of the tumor, and that this immunity is largely peculiar to tumors. Little and Strong (11), Bittner (3), and others, hold that a certain number of factors present in the host are responsible for the fate of the tumor in the host, that the number of these factors can be obtained by determining the percentage of "takes" of transplanted tumor in F2 hybrids between a strain favorable, and one unfavorable to the growth of the tumor. They interpret the difference between normal tissue and tumors, as well as differences between the characteristics of different tumors, as largely due to somatic mutations in normal and in tumor cells.

We have formerly studied the effects of transplanted normal tissues on the white cells of the blood, and found that they correspond to the local reactions around the transplants and that, like the latter, they depend upon the relationship of the organismal differentials of host and transplant (7). In the present investigations we have found that tumor transplants of various kinds behave like normal transplants and that the reactions evoked by both depend upon the relationship of the organismal and, in particular, of the individuality differentials of host and transplant, the differentials of the tumor being essentially the same as those of normal tissues from which they develop. Our results, therefore, confirm the interpretation of Loeb.

Baeslack (1), Murphy and Morton (22, 23), and more recently Lewis (10), have expressed the opinion that the increase in polymorphonuclear leucocytes in the peripheral circulation is a manifestation of a lack of immunity to the tumor. Our experiments show that the animals have become severely anemic at the time when the polymorphonuclear leucocytes have increased; coincidental with these changes in the peripheral blood there progressively develops a profound debility. The association of a secondary anemia with cachexia has frequently been observed in humans with chronic debilitating disease, and Nebenzahl (24) has described such a condition in humans with progressively growing cancer, in whom there was no demonstrable hemorrhage. Similarly, in our experiments there was no obvious bleeding, since animals with ulcerated tumors were discarded. In man it has been reported that a condition similar to pernicious anemia may develop in the presence of a small carcinoma of the stomach; this may perhaps be due to a lack of intrinsic factors. Anemia may also be associated with a small tumor, when, by chance, that tumor erodes a large vessel. It is possible, also, to have a low red blood cell count with a small tumor when the neoplasm

has been produced by the administration of benzol derivatives, owing to the destructive effects of such chemical substances on the bone marrow.

In our experiments, neither a significant anemia nor a definite leucocytosis was found in animals with small tumors; both of these conditions appeared only after the tumor had reached a certain size. They became more pronounced when the tumors were large and the animals markedly cachectic. Tchakhotine (27) believes that the leucocytosis in mice with tumors is due to the effects produced by the protein breakdown which is associated with tumor growth. While we do not accept this interpretation, we do agree with him that the leucocytosis is a function of the size of the tumor, irrespective of whether the tumor is of spontaneous origin or has been transplanted.

We must also consider the possibility of secondary infection in a debilitated animal as a cause of the leucocytosis. While no gross evidence of such a condition was present, the possibility exists that a low grade infection, which did not become manifest, may have occurred in some cases. However, it is not probable that this would apply to so large a number of animals bearing nonulcerative tumors, without any signs of such an infection becoming apparent.

In view of the parallelism between the intensity of the leucocytosis and the degree of anemia in bearers of large tumors, we feel justified in attributing the increase in circulating polymorphonuclear leucocytes to the same factors which were responsible also for the anemic changes.

Summary

Pieces from various kinds of rat and mouse tumors were homoio- and heterotransplanted into rats, mice, and guinea pigs. Benign as well as malignant tumors were used. They all exerted the same effects of increasing the leucocytes in the circulating blood as did normal tissues, the effects of both depending upon the relation of the organismal differentials of host and transplant. Benign tumors reacted in principle in the same way as malignant tumors, although with a slightly diminished intensity. Successive transplantations of tumor pieces likewise acted like those of normal tissues, and tumors and normal tissues interacted in the same way as did 2 normal or 2 tumor pieces successively transplanted.

However, when tumor homoiotransplants reached a large size the animals became debilitated, developed anemia with a secondary hyperplasia of red and white cell elements of the bone marrow, and leucocytosis. This increase in polymorphonuclear leucocytes must be distinguished from the increase in polymorphonuclear leucocytes caused by the transplantation of heterogenous normal or tumor pieces; it is a condition which was not found after homoiotransplantation of normal tissues. This is the only significant difference in the reactions to normal and to tumor tissues on the part of the host which we have observed, and, as stated, we find reasons for attributing this peculiar reaction to tumors, to secondary accidental factors not connected with the organismal differentials of the tissues.

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Cultures in Vitro of Blood Cells, Bone Marrow, and Myocardium From Leukotic Fowls*

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The experiments described here were carried out with Engelbreth-Holm's strain T of chicken hemocytoblastosis. The dried blood which we received represented the 199th passage.

A thorough description of this strain has been given by Engelbreth-Holm and his associates (5, 16). Strain T originated from a spontaneous case of anemic hemocytoblastosis. After the 5th passage the strain divided into 2 branches. One which was almost purely myeloblastic died out after 16 passages. The other, a purely hemocytoblastic branch gives practically 100 per cent takes. The disease usually runs an acute course, but in a small percentage of cases it takes the form of a chronic erythroblastic anemia. Both types are essentially the same. According to Uhl, Engelbreth-Holm, and Rothe Meyer (16) intramuscular and subcutaneous inoculation of leukotic material often causes sarcoma at the site of inoculation, which in most cases is accompanied by hemocytoblastosis.

Our experience with this strain agrees with that of Engelbreth-Holm. We found that inoculation of fresh blood gives 100 per cent takes in chicks and young hens. The disease takes the form of a rapidly progressing hemocytoblastosis, and only in a small percentage of cases appears as a more slowly progressing anemic form.

Experimental Methods

Tissue cultures.—Tissue cultures were made according to Carrel's standard method (3), partly in hanging-drops, partly in flasks (Model D-3:5) as well as in flasks with removable bases as described by Doljanski (4). As culture medium, we used normal chicken plasma diluted with Tyrode solution in the proportion 1:2, to which chick embryo extract was added in varying concentrations. Some of the flask cultures were treated according to the method of "delayed growth" of Fischer and Parker (6). Hanging-drop cultures were transferred every 3rd day, flask cultures every 3rd week.

Histological examinations.—Hanging-drop cultures and cultures in flasks with removable bases were fixed in Carnoy's fluid and stained *in toto* with Giemsa's stain. Cultures in Carrel flasks were fixed in Helly's fluid, embedded in celloidin-paraffin, and cut in series. Sections 5 μ thick were stained in Giemsa's or in hematoxylin-eosin solution.

Inoculation.—The infectivity of cultures was tested by inoculation into chicks. The cultures were introduced into a pocket of the pectoral muscle with a cataract knife or injected intrapectorally with a syringe after fine mincing. In some cases the fluid phase of the culture or the wash-fluid (Tyrode's solution), kept in contact for ½ hour with cultures, was injected intravenously.

Experiments

Cultures of leukotic blood cells.—Blood is taken from the leukotic animals shortly before death, either from the carotid artery or from the heart. Since leukotic plasma coagulates slowly, the addition of anticoagulants may often be dispensed with. After thorough centrifugation the supernatant plasma, which is always colored reddish-brown, is pipetted off, and from the buffy coat which is usually 5 to 8 mm. thick, material for cultivation is removed by means of a pipette. In some experiments coagulation of the buffy coat is brought about by adding embryonic extract. The coagulated buffy coat is removed and cut into small fragments.

The buffy coat of leukotic blood consists almost entirely of stem cells. These are not quite uniform in appearance. They may be divided into 2 groups:

1. Typical hemocytoblasts—large cells, usually round, having a nongranulated, sometimes vacuolated, markedly basophilic cytoplasm and usually a centrally placed, round or almost round vesicular nucleus, with a conspicuous chromatin network and 1 or more nucleoli. These cells are always present in predominant numbers.

2. Very immature cells—large cells markedly irregular in shape, having a grayish-blue, fairly light cytoplasm. The nucleus is pale, oval or kidney-shaped, and almost always eccentric.

Among these primitive cells more mature precursors of red and white blood cells are always found in small numbers. During the course of the disease the number of basophilic and polychromatophilic erythroblasts is gradually reduced. Shortly before death they disap-

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pear almost entirely. The number of promyelocytes and myelocytes is minimal in most cases.

In addition to the stem cells, the buffy coat contains a varying number of polymorphonuclear leukocytes in the lower layers. Lymphocytes and monocytes are usually not recognizable in the buffy coat and when they can be identified among the hemocytoblasts they are always few.

Appearance of cultures.—Six hours after explantation: The coagulum in the neighborhood of the explanted fragment contains a fairly large number of hemocytoblasts lying singly or in small groups, which were introduced into the coagulum with the planting of the cultures. They are similar in appearance to the hemocytoblasts of the leukotic blood (Fig. 1). At this stage only the mature granulocytes migrate actively, forming a circular area around the explanted fragment.

Twenty-four hours after explantation: The granulocytes have advanced far into the plasma clot and many of these cells show signs of degeneration. At this stage there is no noticeable migration of hemocytoblasts, but the central fragment becomes looser and the stem cells which maintain their round shape are pressed somewhat towards the periphery. The hemocytoblasts lying free in the coagulum show some morphological changes. The originally scattered single hemocytoblasts are now frequently arranged in pairs which are distributed in the coagulum like cartilage cells flattened at the sides opposed to each other. Some of the hemocytoblasts in the coagulum increase in size and develop long, sharply defined processes, often at opposite poles, assuming the appearance of a thick spindle. Others become angular or oval; occasionally stem cells are surrounded by a fringe of small pointed pseudopodia; more rarely hemocytoblasts with widely branched pseudopodia are found (Fig. 2).

After 48 hours' incubation: Most of the hemocytoblasts in the outer periphery of the culture have lost their circular outline and have taken on an elliptical, angular, or spindle shape. Those which have remained round usually lie in pairs. Here and there groups of hemocytoblasts are seen consisting of 4, 6, or more cells, divided from one another by narrow, sharply defined, easily visible gaps; these groups are certainly formed by the multiplication of single hemocytoblasts locked in the plasma (Fig. 3). Many cells have 2 nuclei. Occasionally stem cells are seen 3 or 4 times the size of a normal hemocytoblast and possessing 3, 4, or more nuclei (Fig. 4).

Numerous hemocytoblasts show mitotic figures (Fig. 5). One sometimes sees pictures of cell divisions suggesting amitotic processes (Fig. 6). Some of the hemocytoblasts have long, thread-like nuclei, both extremities of which are much thickened; the cytoplasm collects around the expanded ends of these nuclei.

An extensive liquid zone now forms around the original fragment. Small groups of hemocytoblasts are seen lumped together in this zone. Some cells retain their normal appearance, while others show 1 or more vacuoles in their basophilic protoplasm. The contents of the vacuoles frequently push the nucleus to one side and flatten it. At the edge of the plasma coagulum which limits the liquid zone the hemocytoblasts are particularly numerous. Here many stem cells are drawn out and spindle-shaped. The elongated hemocytoblasts tend to be arranged concentrically forming chains of connected cells.

In addition to the stem cells described above there may be found at this stage cells with abundant more or less basophilic cytoplasm and a nucleus like that of monocytes (Fig. 7).

At this stage polyblasts of the macrophage type appear in the cultures, cells which may be seen also in small numbers at the end of 24 hours. They are large and very active ameboid cells, possessing pale, transparent, vacuolated cytoplasm with membrane-like pseudopodia, and a characteristic, pale, and eccentric nucleus, with finely scattered chromatin particles and 1 or more distinct nucleoli (Figs. 8 and 9). The poly-

DESCRIPTION OF FIGURES 1 TO 9

FIG. 1.—Culture of leukotic blood a few hours after explantation. Experiment No. 11042. Giemsa's stain. Mag. × 1700.

FIG. 2.—Hemocytoblasts from culture of leukotic blood 2 to 3 days old, showing cellular prolongations and pseudopodia. Giemsa's stain. Mag. × 1800.

FIG. 3.—Hemocytoblasts from culture of leukotic blood 2 to 3 days old, showing cells in cluster. Giemsa's stain. Mag. × 1900.

FIG. 4.—Hemocytoblast from culture of leukotic blood 2 to 3 days old, showing binucleate cell. Giemsa's stain. Mag. × 1700.

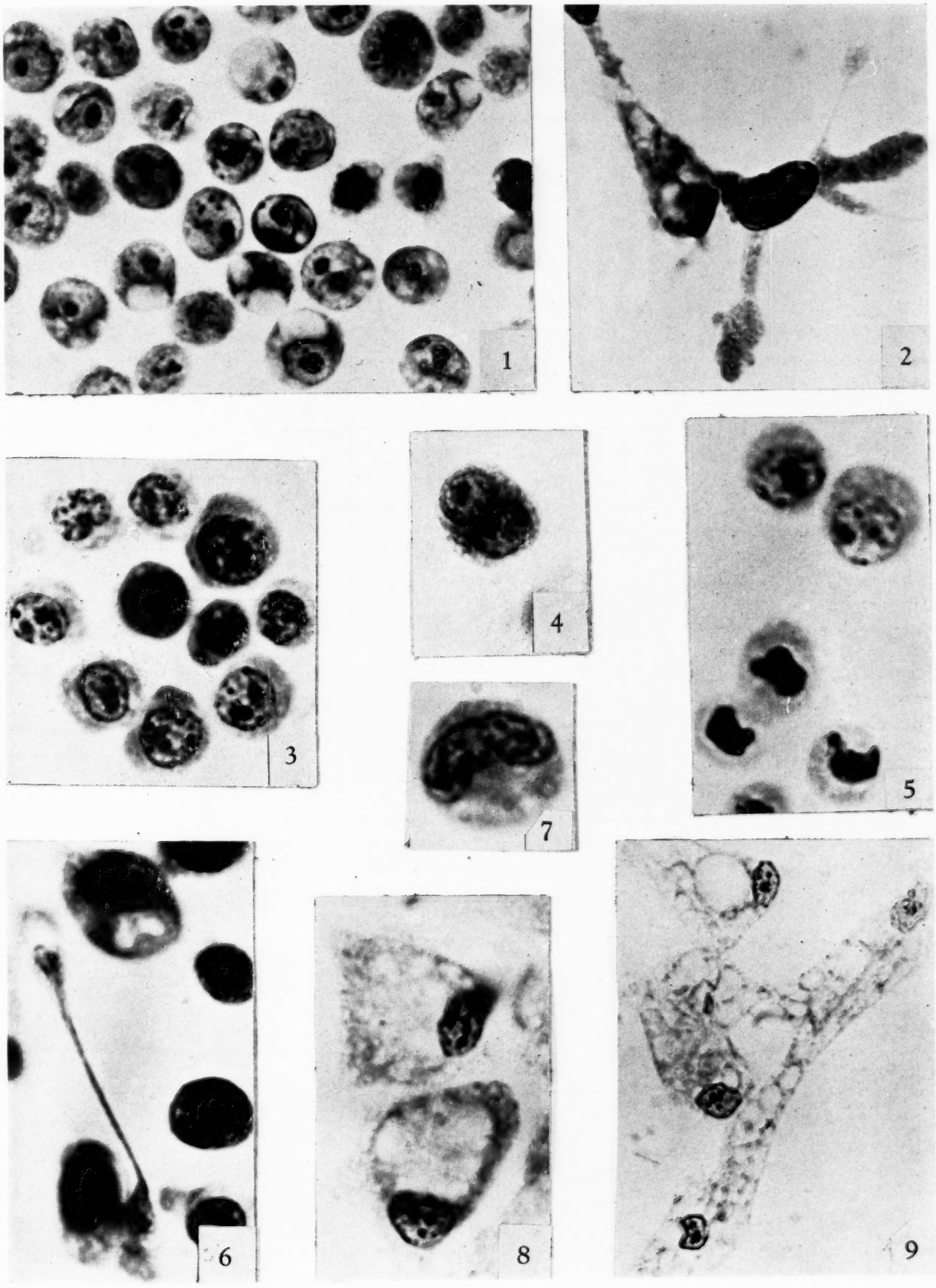
FIG. 5.—Hemocytoblasts showing mitotic figures from culture of leukotic blood 2 to 3 days old. Giemsa's stain. Mag. × 2500.

FIG. 6.—Hemocytoblasts from culture of leukotic blood 2 to 3 days old showing figures suggestive of amitotic division. Giemsa's stain. Mag. × 2300.

FIG. 7.—Hemocytoblast from 2-day old culture of leukotic blood showing monocytic type of nucleus. Giemsa's stain. Mag. × 2400.

FIG. 8.—Large vacuolated cells with eccentric nuclei from a 5-day old culture of leukotic blood. Experiment No. 8885. Giemsa's stain. Mag. × 1700.

FIG. 9.—Cells, similar to those shown in Fig. 8, from 3-day old culture of leukotic blood, showing vacuolization of cytoplasm, elongation of processes, and eccentric position of nuclei. These are regarded as polyblasts of the macrophage type. Experiment No. 11007. Giemsa's stain. Mag. × 1500.



FIGS. 1-9

blasts are often flattened against the mica. During migration the nucleus lies in the posterior part of the cell. Unlike fibrocytes they do not become completely rounded during the process of mitosis (Fig. 10).

In addition to polyblasts which are identical with the polyblastic cells developed in cultures of normal blood cells, cell forms are present showing every transitional step from stem cells to polyblasts. In this process of transition, the cytoplasm gradually loses its basophilia and becomes pale and vacuolated. The cell increases in size and becomes more irregular in shape. The nucleus becomes larger and paler, and assumes the structure of a histocyte nucleus. The changes in the nucleus and the cytoplasm are not always parallel, so that one occasionally sees cells which have the characteristics of a macrophage but possess a typical stem cell nucleus.

The presence of polyblasts and transitional forms between stem cells and polyblasts is regular but not constant.

At the end of 72 hours: There are many stem cells, still unchanged, especially those scattered amongst the proliferating polyblasts. However, most of the stem cells, especially those lying massed together inside the liquid zone, show signs of extensive degeneration. The nucleus gradually becomes small and pyknotic. The cytoplasm loses its basophilia and becomes markedly eosinophilic. Later the nucleus disappears altogether and only a yellowish-pink disc remains, which gradually disintegrates.

In cultures in which polyblasts do not appear, all cells undergo progressive degeneration. In those cultures in which polyblasts appear, the actively multiplying polyblasts form dense layers of flattened cells. They often become spindle-shaped and take on the appearance of typical fibroblasts. These come into close contact with each other, forming long chains or a dense network within which still surviving stem cells are locked (Fig. 11).

According to our observation it is probable that stem cells which have acquired a spindle shape may turn directly into fibroblasts. The remaining ameboid polyblasts of the macrophage type now show particularly well marked phagocytic properties. They are packed with pyknotic nuclei and other cellular debris (Fig. 12).

The further fate of these cell colonies may best be studied in Carrel flask cultures.

The development of leukotic blood cell cultures in flasks does not differ in the early stages from that in hanging-drop cultures previously described. A liquid area forms around the explanted material enclosing numerous hemocytoblasts whose ultimate fate corresponds to that of hemocytoblasts in the liquid zone of hanging-drop cultures. Around this there develops a more or less wide zone consisting of granulocytes and polyblasts among which stem cells are scattered. The granulocytes degenerate rapidly and the number of stem cells decreases from day to day, whereas the ameboid and phagocytic polyblasts multiply and in a short time become transformed into fibroblasts.

In spite of progressive degeneration and transformation into polyblasts, hemocytoblasts do not completely disappear for a considerable time and can persist among the proliferating fibroblasts. Examination of flask cultures, even 2 to 3 weeks old, not infrequently reveals scattered groups of hemocytoblasts in the dense network of spindle cells. The mitoses met with prove that they can multiply among the growing spindle-shaped cells. In some experiments there may be noted a marked rise in proliferating activity of the stem cells in culture. They may be found, not only among the proliferating fibroblasts but also near the periphery, in the form of continually sprouting stem cell colonies. Each of these forms a new liquid zone around which the active multiplication of stem cells takes place. Such secondary colonies not infrequently give rise to small colonies of fibroblasts.

When fibroblast colonies, originating from leukotic blood cells, are transferred into Carrel flasks richly growing spindle cell cultures are obtained which can be maintained without difficulty. The proportion of hemocytoblasts in such colonies steadily decreases, and after 1 to 2 passages they are no longer found. In structure and appearance of cells and in rate of growth, fibroblast cultures, originating from cells of leukotic blood, differ in no way from fibroblast cultures of other origin.

Summary and discussion of results.—The development of leukotic blood cells in cultures may be summarized as follows:

The stem cells multiply rapidly, are slightly motile,

DESCRIPTION OF FIGURES 10 TO 15

FIG. 10.—Polyblastic type of cell from culture of leukotic blood 2 days old, showing pseudopodia and mitotic nucleus. Experiment No. 11002. Giemsa's stain. Mag. × 1100.

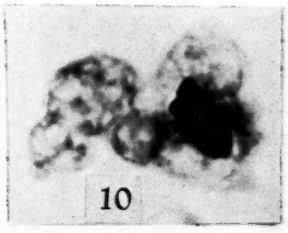
FIG. 11.—Elongated, flattened polyblasts enclosing surviving stem cells, from a culture of leukotic blood 3 days old. Experiment No. 11003. Giemsa's stain. Mag. × 1100.

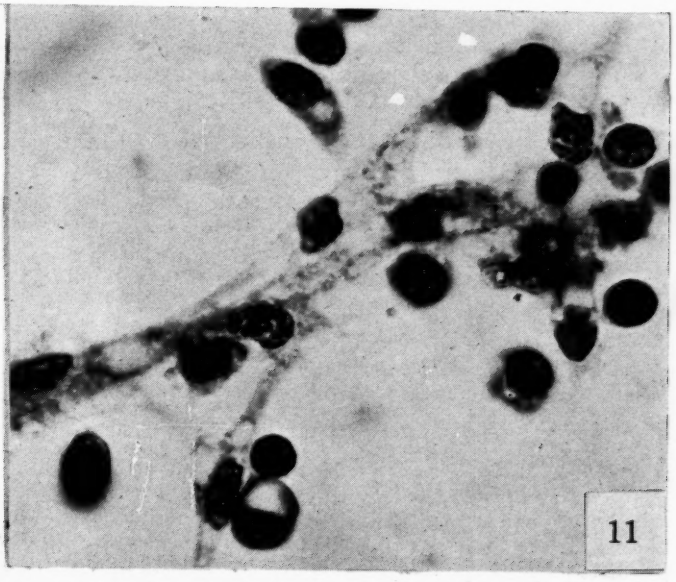
FIG. 12.—Cells from 5-day old culture of leukotic blood, showing ameboid phagocytic polyblasts packed with pyknotic nuclei. Experiment No. 8900. Giemsa's stain. Mag. × 530.

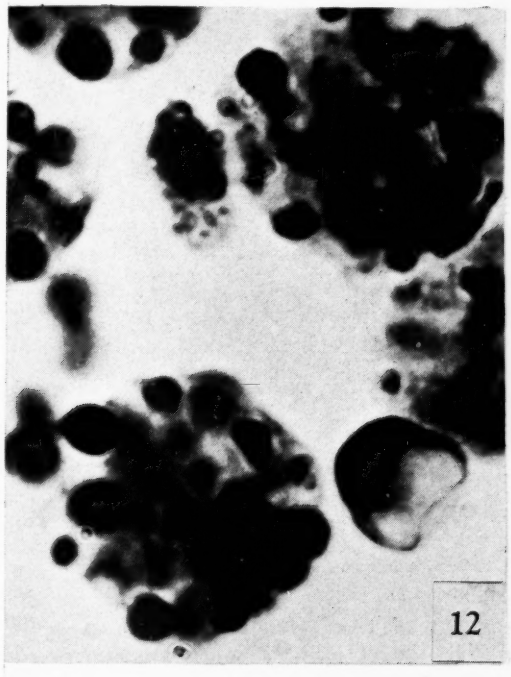
FIG. 13.—Edge of colony of cells in a 2-day old culture of leukotic bone marrow, showing fibroblasts and round cells. Experiment No. 8836. Giemsa's stain. Mag. × 120.

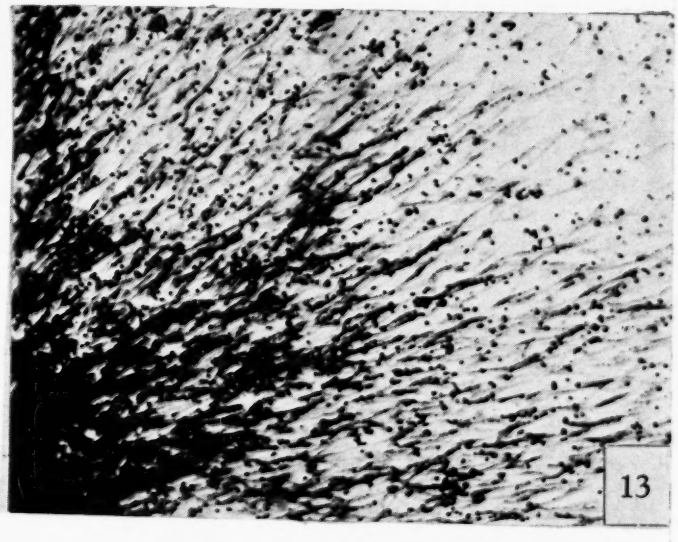
FIG. 14.—Cells from 5-day old culture of leukotic bone marrow, showing round cells among proliferating spindle cells. Experiment No. 9115. Unstained. Mag. × 500.

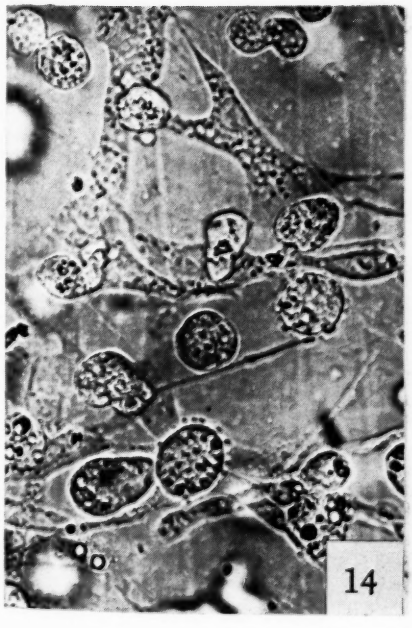
FIG. 15.—Cells from a 2-day old culture of the 4th passage of leukotic fibroblasts from explanted myocardium. Experiment No. 10737. Giemsa's stain. Mag. × 125.

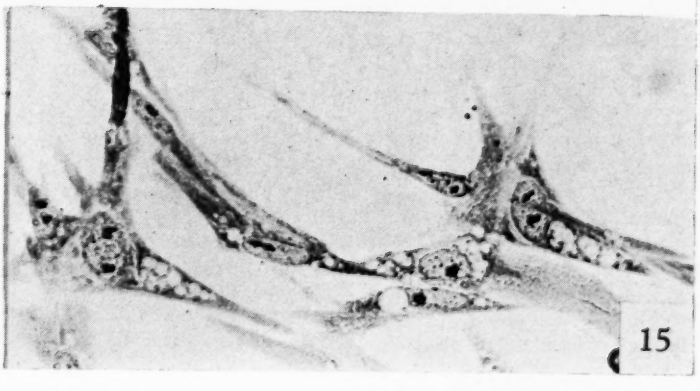












FIGS. 10-15

and liquefy the plasma clot. In the process of cultivation a considerable proportion perishes within a few days; some become transformed into polyblasts; some remain for a long time among the proliferating polyblasts, retaining their characteristics.

The mature granulocytes found in varying number in the original material begin to migrate in the first few hours after explantation and degenerate in the course of the first 2 to 3 days.

It is difficult to identify with certainty lymphocytes and monocytes in the buffy coat of blood removed from a leukotic fowl. It is nevertheless correct to assume, that where these cells are present in the original material, their development does not differ from that known for lymphocytes and monocytes in normal blood; *i.e.,* hypertrophy and transition into polyblast- and fibroblast-like cells (3, 9).

After the granulocytes and most of the stem cells have degenerated, the polyblasts remain the predominant element in the culture and multiply rapidly. Their transformation into fibroblasts goes on continuously, and the culture of cells originating from the blood of a leukotic animal is ultimately transformed into a culture of fibroblasts.

Our observation on the transformation of stem cells agrees with that of most workers who have cultivated immature blood cells (hemocytoblasts, myeloblasts) appearing in human leukemia and who have observed the transition of these cells into polyblasts and fibroblasts.[1]

Thus Awrorow and Timofejewskij (1) showed that myeloblasts from the blood of patients with chronic myeloid leukemia are capable of turning into "hypertrophische" cells and "Ausläuferzellen." Timofejewsky (15) later stated that these terms were meant to designate polyblasts (macrophages) and fibroblast-like cells.

Timofejewsky and Benewolenskaja (14) observed that myeloblasts from a case of acute myeloid leukemia showed a marked tendency to turn into polyblasts, clasmatocytes, and fibroblasts. In a later paper these authors (15), studying 2 cases of acute myeloid leukemia, described the transformation of hemocytoblasts not only into cells resembling polyblasts and fibroblasts but also into plasma cells and multinucleated giant cells.

Hirschfeld (8) described the transformation of myeloblasts from 2 cases of chronic myeloid leukemia, and 3 cases of acute myeloblastic leukemia into cells resembling macrophages and fibroblasts.

Pierce (11) reported that myeloblasts from a case of chronic myelogenous leukemia changed into macrophage- and fibroblast-like cells.

Verrati (17) also observed in blood cultures from 2 cases of chronic myeloid leukemia the development of cells possessing the properties of histiocytes, spindle cells, and giant cells. He believed that the hemohistioblasts and not the hemocytoblasts are the cells capable of undergoing this transformation.

Workers who have cultivated the blood cells of animals with experimental leukosis, give but meagre and contradictory accounts of the morphology of their cultures.

Furth and Breedis (7) reported that in blood cultures from fowl myeloblastosis, several myeloblasts were seen in mitotic division 4 to 9 days after incubation. Cells like myeloblasts persisted 32 days. The cytoplasm of these cells gradually lost some of its basophilia and the cell "resembled both monocytes and myeloblasts."

Bichel (2), who studied the behavior *in vitro* of cells appearing in mouse leukemia, established the survival of "leukosis cells" among added fibroblasts over a period of 5 months. The "leukosis cells" never developed into large phagocytic forms or into fibroblasts.

Ruffilli (13) noted that he observed no transformation of "leukemic cells" in blood cultures from an erythroblastic fowl.

It is not the purpose of this study to discuss the causes underlying the different courses of development which stem cells may undergo. It is possible that "stem cells" include cells with different developmental potentials. These differences may also be due to the variability in culture conditions. Our observations indicate that in the liquefaction zone the stem cells disintegrate particularly rapidly, while in the plasma coagulum they frequently undergo transformation to polyblasts-fibroblasts, and that those scattered among the proliferating fibroblasts may remain unchanged over a long period of time.

We have abstained from discussing the problem of maturation of stem cells *in vitro*. The experimental material at our disposal is not sufficient to allow of definite conclusions as regards maturation of stem cells.

Lymphocytes and monocytes, present in the original material, may give rise to polyblasts which are essential for the future development of the culture. In the end the explanted cells of leukotic blood always give rise to a uniform culture of fibroblasts.

Inoculation of leukotic blood cell cultures.—The results of intramuscular injection of cultures of leukotic blood cells are presented in Table I.

All of the 18 animals injected died of hemocytoblastosis. The disease ran a typical course. The pathological findings in the organs and in the blood were similar to those in the animal infected with the original strain.

We have maintained cultures of blood cells of leukotic animals for 66 days (2nd passage), with full retention of their infectivity. The older original cultures as well as flask cultures of 1st and 2nd passages give as constantly positive results as recently planted cultures. The fact that flask cultures in the 1st and

[1] W. H. Lewis and M. R. Lewis, in extensive studies of white blood cells in cultures, did not observe this transformation. Their observations indicated that lymphocytes, monocytes, and fibroblasts are self-perpetuating types. They stated: "The lymphoblasts undergo mitotic division and move about with characteristic speed and mode of locomotion, but their further transformation has not been observed in our cultures" (p. 372). "Under certain conditions they (monocytes) assume forms somewhat like fibroblasts, but the claim made by some authors that they change into true fibroblasts seems to us very doubtful" (p. 374). See: Cooperation in Research, Carnegie Institute of Washington, Pub. No. 501. 369-382. 1938.

As it has not been possible to communicate with the authors within a reasonable length of time this footnote has been added by the Editor.

even in the 2nd passage retain their infectivity must be especially stressed because the morphological aspect of such subcultures differs from that of the original cell colony. Whereas freshly planted cultures always contain, besides spindle-shaped cells, large numbers of hemocytoblasts, older cultures contain few, if any.

In 1 case, intramuscular injection of a flask culture gave rise to hemocytoblastosis and a spindle cell sarcoma at the site of inoculation. Inoculation of the sarcoma into 3 animals caused hemocytoblastosis in 3; and in 1, in addition, a tumor developed at the site of inoculation.

In later experiments we covered the cultures with Tyrode solution and left them for 30 minutes in the incubator; the Tyrode was then removed and injected intravenously with or without centrifuging. The results of inoculations of chicks with this fluid are given in Table II.

TABLE I: INTRAMUSCULAR INOCULATIONS OF CHICKS WITH FLASK CULTURES OF LEUKOTIC BLOOD CELLS

No. of flask	Passage	Age of culture, in days	Number of chicks inoculated	Number of successful inoculations *	Length of life after inoculation, in days
319	0	7	2	2	14, 11
320	0	14	2	2	14, 58
321	0	21	1	1	17
322	0	22	2	2	77, 22
324	0	33	2	2	28, 14
318	0	38	2	1	14
325	0	47	2	2	14, 67
417	1	49	1	1	.....
415	1	59	2	2	12, 30
445	2	66	2	2	29, 26 †

* Chicks which remained healthy for a period of 3 months were considered negative.
† One chick had, aside from hemocytoblastosis, a tumor (sarcoma) at site of inoculation.

TABLE II: INTRAVENOUS INJECTION OF CHICKS WITH THE WASH-FLUID OF LEUKOTIC BLOOD CULTURES

No. of flask	Passage	Age of culture, in days	Number of chicks injected	Number of successful injections	Length of life after injection, in days
318, 323	0	29	2	2	18, 14
324, 325					
323, 325	0	41	1	1	14
415–417	1	38	1	1	15
415, 423	1	49	2	2	16, 18
424, 442					
443					
323	0	67	1 *	1	17
415–417	2	46	2 †	2	20, 15

* Wash-fluid centrifuged 15 minutes.
† Wash-fluid centrifuged 3 minutes.

Experiments showed that fluid which has been in contact with cultures of leukotic blood cells causes leukosis in animals after intravenous injection.

Summary.—Cultures of leukotic blood cells, kept for 66 days, were shown to be infective during the entire period by inoculation into young chickens. The infectivity of such cultures does not depend on the morphological aspect of the cell colony; cultures rich in stem cells are as effective as cultures which appear to be pure fibroblast colonies. The wash-fluid of such cultures is virulent on intravenous injection.

Cultures of leukotic bone marrow.—We used fragments of bone marrow taken from the upper end of the tibia of a fowl with hemocytoblastosis at the height of its development. The bone marrow used was packed with hemocytoblasts. The lymph follicles still remain here and there.

Appearance of leukotic bone marrow cultures.—The behavior of cultures of leukotic bone marrow *in vitro* corresponds, in principle, to that of normal bone marrow. It differs only in that in the leukotic bone marrow cultures hemocytoblasts predominate.

Examination of a culture of bone marrow 12 hours after explantation shows the original fragment surrounded by numerous, round, markedly basophilic stem cells fairly evenly scattered throughout the coagulum. Their further behavior corresponds exactly to that of the hemocytoblasts from leukotic blood *in vitro*. Like these, they tend to form pairs and later 4, 6, and 8-celled groups. They multiply by mitosis, but figures suggesting amitosis are not infrequent. During the first 48 to 72 hours many of the hemocytoblasts in the coagulum increase in size; many multinucleated forms appear, and spindle-shaped forms as well as cells with more or less branched pseudopodia develop.

An extensive liquid zone now forms round the mother fragment and becomes filled with hemocytoblasts. The cells in the liquid zone degenerate after 2 to 3 days. The stem cells, distributed throughout the coagulum, survive for some time. Many are seen to change into polyblastic cells, as described in the preceding section.

The mature granulocytes migrate at an early period of development of the culture and form the advance line in the zone of growth. They live no more than a few days. The lymphocytes, erythroblasts, and myelocytes are very few in proportion to the stem cells in the original material and are found only occasionally among the cells in the growth area.

The behavior of the bone marrow stroma in cultures of leukotic bone marrow is not different from that of stroma of normal bone marrow. After 24 hours spindle-shaped cells, originating in the bone marrow cell reticulum, may be seen springing up at the edge of the original fragment among the hemocytoblasts. After 2 days the actively proliferating spindle cells form a dense corona and make their way through the

scattered round cells (Fig. 13). In most cases as growth of the culture progresses liquefaction appears. The fibroblasts then form a ring around this zone and gradually fill it.

The growing fibroblasts originating from the stroma are soon joined by those formed as a result of the transformation of the round cells. In this way a homogeneous fibroblast colony is formed. At first such a colony contains round cells, singly, or in groups among the proliferating spindle cells (Fig. 14), but their numbers decrease from day to day. The colony of fibroblasts which results from the transformation of bone marrow cultures differs in no way from a fibroblast culture of other origin.

The development of the leukotic bone marrow culture may be summarized as follows: After the formation of a growth area consisting mainly of large numbers of stem cells and granulocytes, the granulocytes and many stem cells degenerate, and the remaining round cells turn into polyblasts. The polyblasts originating from round cells transform into fibroblasts which mix with those originating from stroma cells. As a result of this process, a uniform fibroblast colony forms.

Inoculation of cultures of leukotic bone marrow.—To test infectivity, cultures of leukotic bone marrow at different stages of development were inoculated into chicks. The total contents of a Carrel flask were finely minced and injected intramuscularly with a syringe. Eight chickens were injected with cultures of 3 series of experiments. Results are shown in Table III. The cultures were from 7 to 30 days old. All except 1 inoculation were successful. The length of life of the chicks after inoculation was from 13 to 66 days.

Summary.—The injections of cultures of leukotic bone marrow into the chicken results almost without exception in leukosis. The leukosis runs the typical course, and animals show all characteristic changes in the blood and viscera. Results of inoculation are independent of the age of the inoculated culture. The cultures 30 days old which are composed of fibroblasts are as effective as young cultures.

Attempt at a continued cultivation of leukotic bone marrow in hanging-drop cultures with the addition of normal bone marrow.—Cultivation of leukotic bone marrow in hanging-drop cultures in successive passages is made difficult by the liquefaction which occurs regularly. In order to overcome the difficulties caused by the marked proteolytic activity of the leukotic bone marrow cultures, we attempted to maintain them, or the leukotic agent contained in them, *in vitro* by adding normal bone marrow fragments to the liquefying cultures at every 2nd passage.

When the residue of a liquefied culture of leukotic bone marrow is brought into contact with a fragment of normal bone marrow, it may be seen that a few fibroblasts sprout from the leukotic tissue together with a small number of round cells. The added normal bone marrow proliferates actively and in its growth completely encloses the leukotic culture. In subsequent passages it is difficult to recognize the original leukotic tissue, for it is quite overgrown by the actively proliferating normal cell elements. In later passages, the elements arising from the leukotic material and identifiable as such completely disappear.

Since cultures of normal bone marrow have a tendency to produce liquefaction of the medium *in vitro,* although this is not as marked as in leukotic bone marrow cultures, fresh normal bone marrow is added to the cultures as long as they show considerable liquefaction. In this way it is easy to obtain a permanent growth of various bone marrow elements, whereby cells of the freshly added normal marrow mix intimately with those explanted and transferred. Such cultures retain pathogenicity during the entire period of cultivation. Inoculation of cultures into the pectoral muscles of 8 chicks was uniformly successful. The cultures were from the 4th to the 10th passage and were grown *in vitro* from 15 to 31 days. The chicks lived from 19 to 77 days after inoculation.

Cultures of myocardium from leukotic fowl.—Fragments of myocardium were removed from a fowl with hemocytoblastosis at the height of the disease. The heart of a leukotic fowl shows no macroscopic changes. Microscopically the heart muscle is seen to be normal; the capillaries always contain immature blood cells.

Heart fragments of a leukotic fowl were cultured in hanging-drop and in Carrel flasks. Cultivation in Carrel flasks was carried out either with addition of 25 per cent embryonic extract or without extract according to the Fischer-Parker method of "delayed growth." Hanging-drop cultures were transferred every 3rd day, flask cultures every 3rd week.

Appearance of leukotic heart cultures.—Following a latent period of 12 to 24 hours the 1st spindle cells appear at the edge of the fragment explanted in Carrel flasks. The growth area spreads from day to day and finally forms a dense corona around the mother fragment. In hanging-drop cultures the explanted heart fragment shows poor growth at the 1st passage; it is only after 2 to 3 passages that active proliferation is established.

The growth area of the explanted heart fragment consists of a network of radially disposed spindle cells. The architecture of such colonies differs in no way from that of cultures of the heart muscle of a normal chicken. The spindle cells forming the colonies have the characteristics of typical fibroblasts (Fig. 15).

The cultures of fibroblasts originating from leukotic heart show the same rate of growth as fibroblasts originating from normal adult heart. They do not

liquefy the plasma medium and their permanent cultivation is simple.

Such cultures in their earlier stages sometimes contain a very small number of round cells; these practically disappear after a few passages.

Inoculation of fibroblast cultures originating from myocardium of leukotic fowl.—Cultures inoculated into the pectoral muscle of the chicks caused typical leukosis. Table III shows the results of inoculations with flask cultures; Table IV the results of inoculations with hanging-drop cultures.

Of a total of 33 chicks inoculated with such cultures 3 developed tumors at the site of inoculation. One chick inoculated with a 31-day old culture developed both a tumor and hemocytoblastosis. The other 2 inoculated with 48- and 43-day old cultures respectively developed tumors without any signs of hemocytoblastosis. From 1 of the latter the tumor was passed to 2 other chicks. Both developed tumors at the site of inoculation as well as fatal hemocytoblastosis.

Not only the cell cultures but also the wash-fluid of Carrel flasks (Tyrode is solution kept in Carrel flask cultures for ½ hour) is infective for the fowl. Centrifugation for 15 minutes and filtration through an ultrafilter (Jena glass filter 5/3) does not remove the pathogenicity of the liquid (Table V).

TABLE III: INTRAMUSCULAR INOCULATIONS OF CHICKS WITH FLASK CULTURES OF LEUKOTIC MYOCARDIUM

No. of flask	Passage	Age of culture, in days	Number of chicks inoculated	Number of successful inoculations	Length of life after inoculation, in days
205	1	37	1	1	20
212–214	2	43	1	1 *	150
239, 241 243, 245	2	45	2	2	21, 59
289, 291 295	3	59	1	1	28
290	3	70	2	2	37, 16
294	3	57	1	1	21
293	3	57	1	0	.....
304	4	70	1	1	14
328	5	81	1	1	19
329	5	106	2	2	15, 24
377	5	95	1	1	15
367	5	99	1	1	33
376	5	107	1	1	44
530, 531 439, 452	7	146	2	1	18
622	9	181	1	1	38

* Killed 150 days after inoculation. Chick had no hemocytoblastosis but a tumor at site of inoculation.

TABLE IV: INTRAMUSCULAR INOCULATIONS OF CHICKS WITH HANGING-DROP CULTURES OF LEUKOTIC MYOCARDIUM

No. of culture	Passage	Age of culture, in days	Number of chicks inoculated	Result of inoculations	Length of life after inoculation, in days
				Experiment A	
8682, 8683	8	25	1	Positive	24
8686–8688	8	25	1	Positive	22
8812–8825	14	44	1	Positive	90
8812–8825a	14	44	1	Negative	..
				Experiment B	
9084–9089	4	14	1	Positive	18
9130–9133	5	21	1	Positive	13
9134–9141	5	21	1	Positive	32
9157, 9159	6	25	1	Positive	17
9156, 9158	6	25	1	Positive	14
9181, 9185/6	7	27	1	Positive	27
9211, 9212	8	31	1 *	Positive	21
9237, 9238	9	34	1	Positive	15
9293, 9295	10	38	1	Positive	15
9318–9320	11	41	1	Positive	55
9434, 9437/8	13	48	1	Positive	†

The tumor was inoculated on 2 chicks. Hemocytoblastosis and tumors at the site of inoculation in both.

* Hemocytoblastosis plus tumor (sarcoma at site of inoculation).

† Killed 78 days after inoculation. Chick had no hemocytoblastosis but a tumor (sarcoma) at site of inoculation.

TABLE V: INTRAVENOUS INJECTIONS OF CHICKS WITH THE WASH-FLUID FROM CULTURES OF LEUKOTIC MYOCARDIUM

No. of flask	Passage	Age of culture, in days	Number of chicks injected	Number of successful injections	Length of life after injection, in days	Remarks
				Experiment A		
368, 369	5	88	1	1	15	
368, 369 346	5	106	2	1	31	Wash-fluid centrifuged
425, 426	6	106	2	2	14, 14	Wash-fluid centrifuged
439–441 452, 453	6	123	2	2	26, 22	Wash-fluid passed through ultrafilter
622, 623	9	181	1	..	.....	Wash-fluid passed through ultrafilter
				Experiment B		
538–543	0	14	2	..	.....	Wash-fluid passed through ultrafilter
538–543	0	30	2	2	13, 16	Wash-fluid passed through ultrafilter

Summary.—Culture of a cell strain is described originating from the heart muscle of a leukotic fowl. The fibroblasts of this strain do not differ from normal fibroblasts. This strain was maintained for 181 days and proved virulent over this period. Inoculation of such fibroblast cultures into fowls caused typical leukosis.

Discussion

Our investigations of cultures of blood cells, bone marrow, and heart muscle of leukotic fowls have shown that as long as the explanted cells remain alive *in vitro,* the cultures retain their pathogenicity. Inoculation of such colonies into fowls causes almost without exception the development of a classical leukosis and occasionally sarcoma. We have succeeded in maintaining the virulence of cultures *in vitro* up to a period of 6 months. Not only the cultures but also the cell free filtrates of the wash-fluid are infective.

The results of our experiments on the cultivation of blood and bone marrow of an animal suffering from hemocytoblastosis agree in the main with those of Furth and Breedis who showed that blood cells, bone marrow, and spleen of a fowl with myeloblastosis remain viable and virulent *in vitro* for a considerable time.

Furth and Breedis (7) cultivated blood cells from a chicken with leukosis produced by "virus I." They reported 2 series of experiments. In the 1st, blood with numerous erythroblasts and a few myeloblasts was used. The authors were able to observe the persistence of myeloblast-like cells over a period of 32 days. The final injection of cells and supernatant fluid removed at this time gave a positive result. In the 2nd experiment they used blood of a chicken with a pure myeloblastic leukemia; myeloblasts were alive during the entire period of observation (26 days). Inoculation at this time was successful. In the same paper Furth and Breedis reported 2 series of experiments on cultures of leukotic bone marrow and 1 of leukotic spleen. In 1 case they used erythroleukemic bone marrow, in the 2nd case myeloblastic. The 1st retained its infectivity *in vitro* for 15 days, the 2nd for 62 days. The spleen cultures remained virulent for 56 days. The bone marrow and spleen were cultivated in Carrel flasks according to the usual technic; the blood cells, on lens paper according to the method of Rous and Beard. The medium used was plasma of leukotic chickens which was replaced after some time by that of normal chickens.

Furth and Breedis (7) advance the opinion that the infectivity of leukotic tissue cultures depends fundamentally on whether they contain primitive blood cells. They see proof of this in the fact that bone marrow and spleen cultures are only virulent when they contain mononuclear blood cells, "presumable myeloblasts." After transformation into fibroblast-like colonies and disappearance of the round cells the bone marrow cultures failed to produce leukosis. Furth and Breedis state:

"In solid cultures of spleen and bone marrow from chickens with myeloid leukemia a symbiotic growth of myeloblasts and fibroblast-like cells occurs. These cultures likewise have the ability to produce leukosis. Fibroblast-like cultures of bone marrow of a chicken with erythroleukosis, not containing leukotic blood cells, failed to produce leukosis."

The results of these investigations led Furth and Breedis to the conclusion that the leukotic virus can survive and multiply only in the presence of primitive blood cells. They see in their experiments a proof of the more general postulate that "oncogenic viruses multiply *in vitro* only in the presence of cells on which they confer neoplastic properties." Furth and Breedis also cited the work of Verne, Oberling, and Guérin (18) as supporting their view.

Verne, Oberling, and Guérin (18) who undertook the first attempts of cultivating the leukotic agent *in vitro,* used the bone marrow of leukotic chickens (strain of Engelbreth-Holm and their own strain). They observed that cultures of leukotic bone marrow in Carrel flasks to which normal bone marrow is added a few times during cultivation are occasionally infective. Out of numerous attempts at inoculation, two 8-day old cultures and one 15-day old culture took. In the hanging-drop colonies, with which the authors also carried out their experiments, the primitive blood cells disappeared completely in the 4th passage, and the culture became transformed into a pure fibroblast colony. Such fibroblast cultures inoculated on the 19th day were not pathogenic. The writers regarded their results with skepticism in view of the small percentage of positive results and because in their experiments no cultures more than 15 days old gave takes. They believed that they were dealing not with a culture but with a limited survival of an apparently very labile leukotic principle.

We omit a discussion of Bichel's (2) experiments in which he obtained virulent cell colonies from cultures of leukotic infiltrations in mice. He was able to observe that virulence of the cultures is conditioned upon the presence of leukosis cells. Inoculation of leukosis cells always produced leukosis, whereas inoculation of the accompanying cells did not produce leukemia. We cannot make use of these results in the problem in which we are engaged, because mouse leukemia, unlike fowl leukosis, has until now been transmitted only by means of cells.

Our observations on the relation between the infectivity of a culture and its cellular composition are not in accord with those cited above. Young cultures of hemocytoblastotic bone marrow with a predominance of primitive blood cells and older cultures which have become transformed into colonies of fibroblasts, take equally well on inoculation. Moreover, cultures of leukotic blood cells which, after repeated subculturing, have become practically entirely transformed into fibroblast colonies, fully retain their pathogenicity.

A study of our material indicates that the infectivity of cultures originating from hemocytoblastotic blood or blood-forming tissue is independent of their cellular composition and of the presence of primitive blood cells.

Furthermore, fibroblast colonies originating from heart fragments of leukotic chicks prove highly virulent on inoculation into animals. The virulence of such a fibroblast strain can be maintained apparently permanently in Carrel flasks as well as in hanging-drop cultures.

This finding confirms that of Ruffilli (13) who observed that fibroblast cultures originating from the heart of an erythroblastotic fowl retain infectivity. He described a cell strain which differed from a normal strain of fibroblasts in that its cells were particularly rich in vacuoles or granules with affinity for neutral red and mitochondria particularly well developed. Besides the spindle cells there were always large and small ameboid cells present. This cell strain remained virulent after

cultivation for 71 days in Carrel flasks and 122 days in hanging-drop cultures (44th passage).

The fact that fibroblast cultures originating from leukotic heart muscle, where from the outset primitive blood cells were present in very small numbers (in the capillaries of the explanted muscle), and which have become practically pure spindle cell cultures in the course of a few passages, remain virulent during the whole period of cultivation, argues for the independence of virulence of the cultures from the presence of "specific" cells. Careful microscopic examination of such pathogenic cell strains in later passages fails to reveal the presence of primitive blood cells.

The pathogenicity of cultures of leukotic tissue appears therefore to be preserved by the proliferating spindle cells alone. Experiments with fibroblast cultures originating either from blood cells, bone marrow, or myocardium of a hemocytoblastotic fowl, support the view that an indifferent mesenchyme cell proliferating *in vitro* enables the pathogenic principle contained in leukotic tissue to survive.

The maintenance of undiminished virulence during numerous passages, in spite of washing of the cultures every 2 to 3 days and in spite of constant change of media, can be explained by assuming an increase of the pathogenic principle.

The agent which possesses the property of selectively stimulating the continuous proliferation of primitive blood cells in the body apparently requires for its "multiplication" the presence of nonspecific mesenchyme cells alone.

The possibility that individual primitive blood cells persist in apparently pure cultures of fibroblasts over a period of more than 6 months without being observed is highly improbable. It cannot, however, be theoretically excluded so long as we confine ourselves to experiments with cultures originating from leukotic tissues in which primitive blood cells are always present. Other experimental methods must be used in order to solve the problem of the relationship between leukotic agent and various cell types.

Summary and Conclusions

1. The progressive development of cultures of leukotic blood cells and the fate of hemocytoblasts in cultures is described. In the course of cultivation these cultures are practically freed of blood cells and the leukotic blood cells are finally transformed into fibroblasts.

2. Cultures of leukotic blood cells remained virulent during the entire period of cultivation (66 days). Of 18 chicks which were inoculated with such cultures, at different stages of cultivation, 17 died of leukosis. The wash-fluid of cultures of leukotic blood cells also proved virulent on intravenous injection. All of 9 chicks injected with wash-fluid from cultures 29 to 66 days old, died of leukosis.

3. There is no relation between the infectivity of cultures originating from cells of leukotic blood and their morphological character; cultures which appear to be pure fibroblast colonies are as infective as cultures rich in stem cells.

4. Cultures of leukotic bone marrow are ultimately also transformed into colonies of fibroblasts.

5. Cultures of leukotic bone marrow cultivated for 30 days were infective throughout this period. Of 8 chicks inoculated with leukotic bone marrow cultures from 7 to 30 days old, 7 died of leukosis. The results of inoculation were independent of the age of the culture and its stem cell content.

6. A cell strain is described which originated from leukotic bone marrow and was cultivated by the addition of fragments of normal bone marrow for 31 days in 10 passages. Eight of such cultures inoculated after 15 to 31 days of cultivation proved infective.

7. Cell strains originating from heart muscle of a leukotic fowl are described. One was cultivated 181 days in flasks, the other 48 days in hanging-drops. The fibroblasts of such strains did not differ in any way from those of normal fibroblasts.

8. Cultures of fibroblasts originating from heart muscle of leukotic chicks were virulent during the entire period of cultivation. Of 19 chicks inoculated with flask cultures from 37 to 181 days old, 16 died of hemocytoblastosis. Of 14 chicks inoculated with hanging-drop cultures 12 died of hemocytoblastosis. The wash-fluid of such cultures proved infective also when passed through an ultrafilter. Of 12 chicks injected intravenously with wash-fluid, from cultures 14 to 181 days old, 8 died of leukosis.

9. The agent used in these studies (strain T of Engelbreth-Holm) produces both leukosis and sarcoma. Several chickens inoculated with tissue cultures developed sarcoma at the site of injection.

10. These experiments indicate that the agent of fowl leukosis is capable of remaining active and of increasing in the presence of nonspecific mesenchymal cells (fibroblasts).

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The Influence of Terminal B Avitaminosis with Attending Low Body Temperature Upon the Growth Characteristics of Sarcoma 180*

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It is well known that there is a fall in body temperature in the terminal stage of B avitaminosis. This phenomenon has been observed repeatedly in those of our experiments on the nutrients essential to tumor growth (mouse sarcoma 180) in which the B complex has been withheld (1). We have used avitaminosis in the experiments here reported to produce a condition of lowered body temperature for a continuous period exceeding the duration of time it was found possible to maintain tumor-bearing mice in this condition by lowered environmental temperature (2, 3). The study, therefore, is concerned with the influence upon tumor growth of B avitaminosis, with attending restricted caloric intake and lowered body temperature, emphasis being placed upon the accurate measurement of the degree and period of lowered body temperature, and the possibilities of its prolongation without sacrifice of the animal

Experimental

Experiment I.—Thirty mice of the Marsh-Buffalo strain, 25 to 27 gm. in weight, were divided into 2 experimental groups by random sampling. One group was placed on a standard calf meal diet containing all essential nutrients in adequate amounts. Another group received our synthetic diet No. 5 (1), which is completely vitamin-deficient. On the 9th day of this regime all mice were inoculated subcutaneously with sarcoma 180. Inguinal skin temperatures were taken by thermocouple twice daily, in the morning and evening. The inguinal skin temperature of the controls on the adequate diet ranged from 35.2 to 37° C. On the morning of the 17th day after inoculation, the mice on the vitamin-free diet showed lowered skin temperatures (as low as 27° C.), which, however, rose somewhat during the day. There were 8 mice in which the skin temperature was continuously below 33° C. for periods ranging from 24 to 72 hours, which survived. After 31 days on the vitamin-free diet, 4 mice had succumbed. On the 32d day, the surviving mice were placed on a diet rich in vitamin B. There were 6 of the survivors whose skin temperature was below 33° C. for a continuous period ranging from 60 to 72 hours. The mean temperature in ° C. for these mice follows. The days are recorded from the date of inoculation.

	20th day		21st day		22d day		23rd day
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
Control mice	35.4	36.5	36.2	35.7	36.5	37.0	35.3
Mice with B avitaminosis	32.9	31.6	30.2	32.5	28.9	31.8	25.8

In Table I are recorded the mean tumor diameter and mean body weight changes for the 6 mice during the period of avitaminosis and the recovery period. It will be noted that on the 10th day of tumor growth, which was the 19th day on the vitamin-free diet, the size of tumors of the control and experimental mice is the same. In the ensuing period the tumors of the mice on the vitamin-deficient diet ceased to grow, while the control tumors more than doubled in diameter. In the recovery period, the tumors of the mice which had been on the deficient diet grew to the same degree as did the controls originally. The B deficiency, with attending low body temperature and caloric restriction, while it completely arrested growth, had no permanent effect upon the tumor growth characteristics. We have recorded only the data for mice which showed a temperature below 33° C. for a continuous period. The data for the vitamin-deficient group as a whole were not significantly different from these and therefore have not been recorded. The mice with a continuous period of lowered temperature responded in the same way as did mice with intermittent periods of low temperature.

Experiment II.—The diet regime, inoculation procedures, and temperature measurements were the same as in Experiment I. Beginning on the 14th day after inoculation, lowered body temperatures were recorded. Four mice in the group on the deficient diet showed inguinal skin temperature below 33° C.—as low as

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21° C.—for periods ranging from 80 to 96 hours. These mice were returned to the standard calf meal diet at the end of this low temperature period. The other mice on the deficient diet showed transient low body temperatures or succumbed when attempts were made to continue B avitaminosis. The mean temperatures for the 4 mice which had continued low temperatures were as follows. The days are numbered beginning with the 1st day of lowered temperature.

1st day		2d day		3d day		4th day	
A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.
28.7	32.8	28.5	31.3	25.5	30.7	23.2	32.9

In Table I are recorded the mean tumor diameter and mean body weight changes for the 4 mice during the period of avitaminosis and for the recovery period.

In this experiment, the tumors of the mice on the deficient diet were smaller the 10th day of tumor life than those of the controls on the adequate diet. There was a very slight tumor growth during the period of avitaminosis. The growth rate during the recovery period parallels the growth rate of the controls during a comparable period of tumor size.

The behavior of the tumors of the 6 mice in Experiment I was remarkably uniform. During the period of vitamin deficiency the growth of all 6 was completely arrested; during the recovery period all resumed rapid growth. The behavior of the tumors of the 4 mice in Experiment II was equally uniform. Since we have observed several hundred mice with avitaminosis and lowered body temperature, and since the behavior of the tumor was always the same, the experiment will not be repeated. The purpose of these experiments, as stated, was the accurate measurement of the degree and period of lowered body temperature.

TABLE I: GROWTH OF SARCOMA 180 IN MICE DURING PERIOD OF B AVITAMINOSIS (WITH LOW BODY TEMPERATURE) AND DURING SUBSEQUENT RECOVERY PERIOD

	Mean tumor diameter in mm.					
	Vitamin B deficiency period			Recovery period		
	Days after inoculation			Days after inoculation		
	10th	15th	23d	31st	36th	41st
Experiment I						
Control on standard diet						
Tumor diameter in mm.	7	12	16 ± 0.9	.....	.....	.....
Body weight change in gm.	+ 1.1	+ 1.7	+ 1.7	.....	.....	.....
Avitaminosis with low temperature						
Tumor diameter in mm.	6 ± 0.7	8 ± 0.8	8 ± 0.6	13 ± 1.0	18 ± 1.7	18 ± 1.7
Body weight change in gm.	— 7.0	— 8.6	— 10.8	— 4.0	— 1.1	0
	Days after inoculation			Days after inoculation		
	10th	15th	20th	25th	30th	35th
Experiment II						
Control on standard diet						
Tumor diameter in mm.	10 ± 0.4	13.5 ± 0.6	15 ± 0.8	16.7 ± 0.8	.....	.....
Body weight change in gm.	+ 1.6	.....	.....	+ 3.4	.....	.....
Avitaminosis with low temperature						
Tumor diameter in mm.	3	3.7	5	11.8	15.5	18.0
Body weight change in gm.	— 6.0	— 7.8	— 8.4	— 1.8	+ 0.7	.....

DISCUSSION

It is well known that tumor cells are resistant to low temperatures *in vitro* and resume growth on transplantation. Our studies have shown that, in the mouse, sarcoma 180 and the Marsh-Buffalo adenocarcinoma will withstand a body temperature below 20° C. for 24 hours or a series of such exposures for 7-hour periods, without suffering a change in growth characteristics. In the present study sarcoma 180 was found to resist a continuous period (60 to 98 hours) below 33° C. (33° C. to 21° C.) without suffering a change in growth characteristics. Lucké (4) and Lucké and Schlumberger (5) have shown that in the frog tumor growth continues during hibernation. There is, therefore, little evidence that the rapidly growing cell is more sensitive to lowered body temperature. Our experiments with the mouse indicate that it is less sensitive, for while the body as a whole suffers atrophy, tumor growth merely ceases or proceeds at a retarded rate. In the present experiments, not only is lowered body temperature imposed, but also caloric restriction and vitamin deficiency. The conditions were obviously sublethal. The importance of the whole study rests on the observations that tumor growth is resumed on return to the normal nutritional state and that the growth characteristics were not changed by the combined factors imposed.

SUMMARY

By producing B avitaminosis in mice of the Marsh-Buffalo strain, the inguinal skin temperature was

reduced 2.5° to 16° C. below normal for periods ranging from 60 to 98 hours.

The growth of subcutaneous transplants of sarcoma 180, which was completely arrested or markedly retarded during the period of avitaminosis with attending lowered body temperature, was resumed at a normal rate on return of the mice to a normal nutritional state.

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The Effects of Reduced Temperatures Upon Growth and Metabolic Changes of Sarcoma 180 Grown in Vivo

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In previous experiments on the effects of irradiation on metabolic processes (5-7) a decrease in oxygen uptake and in aerobic and anaerobic glycolysis of excised tissues, normal and malignant, was observed when these were kept sealed and under sterile conditions at room temperature of about 22°C. In tissues kept in the incubator at 37.5°C. for only 4 to 6 hours a still greater decrease in these processes occurred. Tissues of the same type, however, retained their normal metabolism when kept in an electric refrigerator at about 4°C. for the same length of time or longer, up to 24 hours.

That basic chemical reactions which maintain living processes in the organism can be suspended by low temperatures and yet life be preserved for a certain period of time is a well established fact. The observations mentioned above, however, were made on tissues or organs removed from the body, deprived of the blood and lymph supply as well as other factors controlling the life processes. Comparatively few observations are available in the literature on the effects of low temperatures on living processes *in vivo.* The recent observations made by Fay and Smith (2, 8), who conclude that cold exerts a harmful effect upon the proliferation of human tumors within the organism, prompted investigation of this important problem in tumor-bearing animals.[1]

Experimental Procedure

The Crocker mouse sarcoma 180 was chosen for these experiments for the following reasons: (a) A strain of mice, CFX (Carworth Farms) which produces 100 per cent takes when injected with this tumor, with no spontaneous regressions, was available. (b) Since this tumor had been used in previous experiments (5-7), data were at hand as to the rate of growth *in vivo,* as well as of the metabolic changes *in vitro,* affording some basis for comparisons.

Mice bearing tumors 8 to 10 days old were used in most instances, since neoplasms of this type and age are in the most active stage of development, and their increase or decrease in size can be easily followed. The first task was to determine the lowest degree of temperature and the period of exposure the animals could stand and still survive. A certain number of normal as well as of tumor-bearing mice was sacrificed for this purpose. A temperature of about 5-7°C. for a period of 8 to 24—and in a few instances 48—hours, was found to be the limit of endurance.

The weight of the mouse proved to be a factor in resistance to low temperatures. Older, well fed mice endured a lower temperature than those of lighter weight. Mice weighing less than 20 gm., for example, could barely stand a temperature of 6-7°C. longer than 8 to 12 hours, while mice of 25 to 30 gm. remained alive for 24 to 48 hours at this temperature range. This may be due to an increased toughness of the skin in the older animals. Mice about 3 months old, weighing 25 to 30 gm., were used in most experiments, since the tumor grows well in animals at this age.

After the size of the tumor and the body weight were determined, the mouse was put in a metal can (commercial coffee can), the cover of which was perforated with air holes. A few cubes of dog chow and a container with water were put in each can and the cans were placed in an electric refrigerator at a regulated temperature. The mice consumed a certain amount of food while kept at stated temperatures but, despite the food consumption, a loss of weight resulted depending upon the time the animal remained in the refrigerator. Among mice weighing 25 to 30 gm. kept between 5 and 7°C. for 18 to 24 hours the average loss of weight was about 8 to 10 per cent. This may be due to loss of water as a result of the reduced environmental temperature and the decreased food consumption. The rectal temperatures of the mice were determined before exposure to cold and immediately after their removal from the refrigerator, by means of a thermocouple with an accuracy of 0.1°C. (Tycos Dermatherm, Taylor Instrument Co., Roch-

[1] After this paper was completed, an article by Bischoff and Long (1) dealing with similar experiments appeared. The results obtained by these authors are being taken into consideration in the final discussion and conclusion of this paper.

ester, N. Y.). A special device was constructed for insertion of the thermocouple junction into the rectum. Among normal mice the rectal temperatures varied between 36.5 and 38°C. This wide variation is thought to depend upon the nervous condition of the animal while the determination was being made and upon the environmental temperature. More uniform temperatures were observed after removal of the animals from refrigeration.

The duration of each treatment depended upon various conditions. When a mouse was shivering intensely or was almost motionless, apparently near death, it was removed from the refrigerator, and refrigeration was resumed when the behavior appeared normal. According to the apparent condition of the mouse the treatment was extended from 8 to 12 to 24, and in some instances, to 48 hours. For the evaluation of the effect of reduced environmental temperatures 2 different methods were used: (a) measurements of the growth rate of the tumor *in vivo* and (b) measurements of the metabolic rate of the excised neoplastic tissue. Three dimensions of the tumors were measured by means of a vernier caliper before the mice were put in the refrigerator, immediately after their removal, and a day or two after they had remained at the usual temperature of the animal house. The metabolic rate of the tissues, suspended in P-Ringer solution containing 0.2 per cent glucose at pH 7.4, was measured in a Barcroft-Warburg manometric apparatus at 37.5°C.

To obtain quantitative results for dissolved gaseous and solid metabolites, perfect diffusion must be secured; *i.e.*, all portions of the tissue slices must respire. Hence their thickness is of great importance. Our own device, an instrument for cutting slices of living tissue of uniform thickness has proved satisfactory for experimentation with firm tissues, particularly human tumors, as scirrhous cancer.

This instrument consists of 2 "Ever-Ready" razor blades, a metal frame, and 2 steel plates. The 2 steel plates clamp on to each side of the metal frame by means of screws and wing-nuts, sections of the frame and plates having been cut away to form 2 grooves. These 2 grooves are separated throughout their length by a slightly tapered tongue. The backs of the razor blades are inserted into these grooves by a motion toward the handle. The tongue, beside separating the blades, also provides for their proper spacing. With tightening of the wing-nuts the lower edges of the 2 plates lock the blades against the tongue, aligning the cutting edges with uniform and parallel spacing. The distance between the sharp edges of the blades determines the thickness of the tissue slice. Identical and uniform sections of the desirable thickness of 0.3 mm. are insured by the use of this instrument.

During the course of these experiments, the newly devised instrument was used when the sarcoma was encapsulated and firm; when the tumor was soft it was cut with fine sharp scissors.

For the determination of lactic acid in the substrate, the method of Friedemann and Graeser (3) was employed. The metabolic rate *in vitro* of sarcoma 180, both under conditions of refrigeration and in the controls, was observed for 2 hours in each experiment. The water content of each tumor, experimental and control, was determined in a separate sample by drying a certain amount of the tissue in an electric oven at 110°C. to a constant weight.

Experimental Results

Growth rate.—Of the 95 tumor-bearing mice used in these experiments, only those which survived the longest period of exposure to cold will be discussed. The observations are recorded in Tables I and II.

In 5 mice (Nos. 4, 6, 7, 10, and 13) subjected to various periods of refrigeration the tumors disappeared. With mouse No. 4 refrigeration was begun with a period of 8 hours and extended to 24 hours. The tumor gradually disappeared during 2 weeks' refrigeration. In Experiment 7, the tumor, 8 days old, was small. It increased in size during the first period of treatment, attaining linear dimensions of $1.0 \times 0.9 \times 0.8$ cm. It disappeared during subsequent intermittent periods of refrigeration. Similar observations were made in Experiment 10. Refrigeration was begun 8 days following tumor inoculation. The tumor increased in size during treatment, from $0.6 \times 0.6 \times 0.5$ cm. to $1.2 \times 0.9 \times 0.7$ cm. Toward the end of a later treatment of 36 hours the mouse was found scarcely breathing; it appeared cachectic. Recovery was complete, however, after 24 hours at room temperature of about 22°C. A gradual diminution and eventual disappearance of the tumor occurred. Gradual disappearance of a tumor occurred also in Experiment 13. The tumor remained unchanged in size during the first part of refrigeration but later disappeared rapidly.

In Experiments 1, 2, 3, 11, 14, and 17, the tumors either remained unchanged in size or increased slightly during refrigeration. After about 2 weeks mice Nos. 1, 2, and 3 died during refrigeration. In mouse No. 11 no significant change in tumor size was observed following 112 hours of refrigeration. This tumor was removed for the determination of respiratory changes. Nor was any apparent change in the size of the tumor in Experiment 14 noticed during a period of 92 hours of intermittent refrigeration, which was discontinued because of ulceration of the tumor. A slight decrease in tumor size occurred in Experiment 17 after a period of refrigeration of 98 hours. This tumor was also used for the determination of the metabolic rate. The remaining experiments included in this table, Nos. 5, 8, 9, 15, and 16, serve only as examples of many other observations made on the 95 tumor-bearing mice used in the course of these studies. As will be noticed, an increase in the size of the tumors occurred despite

TABLE I: BEHAVIOR OF SARCOMA 180 IN MICE * KEPT IN ENVIRONMENTAL TEMPERATURES OF 5-7° C. FOR VARIOUS PERIODS OF TIME

No. of experiment	Size of tumors in cm. Before refrigeration	Size of tumors in cm. After refrigeration	Total hours of refrigeration	Remarks
1	1.1 0.7 0.6	1.1 0.8 0.8	72	Tumor growth arrested during intermittent refrigeration. Mouse found dead in refrigerator after total treatment of 72 hours applied in periods of from 8-24 hours.
2	1.0 0.7 0.7	0.8 0.8 0.6	72	Slight decrease in tumor size following total refrigeration of 72 hours, applied intermittently. Mouse died during refrigeration.
3	1.4 1.1 1.0	1.3 1.0 0.9	54	Slight decrease in tumor size. Animal died during a 24-hour refrigeration.
4	1.1 0.8 0.6	Disappeared	115	Mouse refrigerated intermittently, 8-24 hour periods, during 2 weeks. Tumor gradually disappeared.
5	1.6 1.3 1.0	Ulcerated	58	Tumor size increased during refrigeration. Experiment discontinued because of ulceration of tumor.
6	0.8 0.6 0.6	Disappeared	79½	Refrigeration begun 8 days after inoculation. Tumor gradually disappeared.
7	0.75 0.60 0.50	Disappeared	221	Tumor size increased during first period of refrigeration to 1.0 x 0.9 cm. Gradually disappeared during later refrigeration. Mouse still alive.
8	1.3 0.7 0.7	1.9 1.2 0.9	80	Tumor size increased during course of refrigeration but was smaller than control tumors.
9	1.0 0.6 0.6	1.6 1.3 1.2	127	Tumor size increased in spite of refrigeration, but less than controls. Tumor used for determination of metabolic rate.
10	0.6 0.6 0.5	Disappeared	110	Tumor increased to 1.2 x 0.9 cm. during first treatment period. Animal found scarcely breathing during 36-hour refrigeration; recovered during 24 hours at room temperature. Tumor gradually disappeared afterwards.
11	2.0 1.3 0.9	2.2 1.5 1.3	112	Tumor size increased slightly during 2 weeks' intermittent refrigeration. Increase smaller than in control.
12	0.8 0.7 0.7	1.7 1.2 0.9	118	Tumor size increased despite 118 hours refrigeration. Mouse died during 48-hour refrigeration.
13	0.8 0.6 0.6	Disappeared	71	Tumor remained unchanged in size during 1st refrigeration period but disappeared rapidly afterward.
14	1.7 1.4 1.0	1.5 1.2 1.0	92	Slight decrease in tumor size. Refrigeration discontinued because of ulceration of tumor.
15	0.9 0.6 0.5	2.0 1.4 1.0	105	Slow increase in size during course of refrigeration. Tumor used for determination of metabolic rate.
16	1.5 1.1 0.9	2.2 1.6 1.2	104	Increase in tumor size. Increase small compared with control tumors.
17	1.4 1.0 0.8	1.2 0.6 0.6	98	Slight decrease in tumor size after total of 98 hours' refrigeration. Tumor used for determination of metabolic rate.

* The rectal temperature of mice varied between 29-31° C.

TABLE II: OXYGEN CONSUMPTION, RESPIRATORY QUOTIENTS, AND AEROBIC GLYCOLYSIS OF EXCISED SARCOMA 180 IN VITRO IN 0.2 PER CENT DEXTROSE RINGER-PHOSPHATE SOLUTION BUFFERED TO PH 7.4 AT 37.5° C. OF REFRIGERATED AND CONTROL SARCOMA 180 GROWN IN VIVO *

	Refrigerated in vivo						Control				
No. of experiment	QO_2 in 1st hour	QO_2 in 2nd hour	R. Q.	Gamma lactic acid per 1 mgm. tissue—2 hours	Per cent H_2O in sarcoma 180	Remarks	QO_2 in 1st hour	QO_2 in 2nd hour	R. Q.	Gamma lactic acid per 1 mgm. tissue—2 hours	Per cent H_2O in sarcoma 180
1	0.243	0.261	0.972	2.2	76.4	Mouse kept for 24 hours at 5° C.	0.670	0.647	0.926	5.3	78.8
2	0.251	0.281	0.763	2.1	79.3	Mouse kept at 5° C. for 17 hours	0.287	0.214	0.717	1.1	76.16
3	0.220	0.315	0.800	3.2	76.5	Mice kept at 6° C. for 24 hours	0.367	0.326	0.89	2.0	76.92
4	0.332	0.374	0.813	2.7	76.6	Mouse kept at 7° C. for 18 hours	0.321	0.305	0.820	2.9	76.14
5	0.253	0.245	0.710	2.0	79.5	Mouse kept at 6° C. for 20 hours	0.425	0.410	0.91	4.4	76.18
6	0.244	0.244	0.768	1.3	79.9	Two mice kept at 6° C. 2 days in succession for 12 hours.	0.210	0.198	1.10	1.2	81.8
7	0.140	0.120	0.610	0.2	80.0	Mouse kept at 6° C. for 24 hours. Found dead in refrigerator.	0.350	0.310	0.924	2.1	81.9
8	0.340	0.340	0.690	3.3	75.7	Mouse kept at 6° C. for 24 hours	0.470	0.410	0.76	2.5	80.8
9	0.288	0.255	1.270	2.2	78	Mice kept at 7° C. for 48 hours	0.280	0.270	0.827	2.9	80.2
10	0.195	0.240	0.730	3.1	79.3	Mouse kept at 6° C. for 22 hours	0.270	0.260	1.10	2.4	82.1
11	0.320	0.266	1.050	4.2	...	Mouse kept at 6° C. for 18 hours	0.450	0.301	0.990	3.4	...
12	0.350	0.390	1.000	3.2	...	Mouse kept at 5° C. 3 days in succession for 9 hours.	0.260	0.200	0.890	2.9	79.5
13	0.348	0.208	0.910	2.1	79.9	Refer to Exp. 9, Table I. Mouse refrigerated for 127 hours.	0.245	0.215	0.780	3.4	81.2
14	0.179	0.210	0.806	1.2	78.5	Refer to Exp. 17, Table I. Mouse refrigerated for 98 hours.	0.289	0.178	0.810	...	79.9
15	0.186	0.290	0.680	2.2	81.5	Mouse kept at 5° C. for 42 hours	0.320	0.260	0.830	3.0	82.0
Average of 15 cases	0.260	0.269	0.803	2.3	...		0.344	0.297	0.884	2.8	...

* For each experiment 14 vessels were used and the average results calculated on wet weight are recorded.

prolonged exposure to a low temperature at intermittent periods. In Experiment 9, for example, the tumor practically doubled in size despite a total refrigeration of 127 hours.

A high mortality rate among the refrigerated mice was observed. Death occurred in the majority of instances during refrigeration. Other animals died a day or two afterward. Histologic study of the lungs, liver, and kidneys did not reveal any pathologic changes. A histologic analysis was also made of several tumors following longer periods of refrigeration in comparison with sections of control tumors of a corresponding age. This was done particularly with tumors which were used for the determination of the metabolic rate. Since no apparent change in the histologic structure of the tumors was noticed, as compared to the controls, the pictures are not reproduced here, although they are available.

Metabolic rate.—The data as to respiration and aerobic glycolysis included in Table II were obtained under the following conditions: Tumors developing 12 to 16 days after inoculation were used in all the experiments. The tumors were extirpated under aseptic precautions immediately after removal of the mice from the refrigerator. The more viable, peripheral parts of the tumors were usually selected, and the central part discarded. The same procedure was applied to the control tumors. Certain amounts of tissue, weighed on a torsion balance, were placed in vessels containing ice-cold P-Ringer solution. With our improved Barcroft-Warburg technic (5-6) the time elapsing between the removal of the tumor from the animal and the first manometer reading of the respiration *in vitro* was about 50 minutes. The tissue was kept on ice during the technical procedure.

The QO_2 of the sarcoma of individual refrigerated mice varied from 0.18 to 0.33 in the 1st hour and from 0.15 to 0.39 in the 2nd hour of observation, with the single exception of Experiment 7, in which the figures were lower. The tumor in this instance was obtained from a mouse which died during a 24-hour treatment in the refrigerator. This case is included in order to show that tumor tissue removed from a dead animal is able to respire, though at a lower rate. The majority of the mice used in these experiments were kept at temperatures between 5 and 6°C. for periods of 17 to 24 consecutive hours. In Experiment 9 the refrigeration period was 48 consecutive hours. In Experiments 12, 13, 14, and 15 daily refrigeration periods of 7 to 9 hours were employed, the total ranging from 27 to 127 hours. When the respiration rates for the individual experiments are compared, no significant differences are observed in relation to duration of refrigeration. The results lie within the same range whether this amounted to 12 to 24 and 48 hours continuously or to 127 hours intermittently (Experiment 13). The same can be said about aerobic glycolysis. The lactic acid production, expressed in γ per mgm. tissue, varied in individual cases, but not in relation to the duration of refrigeration.

Comparing the results obtained from individual tumors in the experimental series with the control tumors, we find similar variations in each group. It is of interest that there was no marked decrease in respiration of the experimental tumors *in vitro* during the 2nd hour of observation, thus proving the viability of the cells in spite of refrigeration. Indeed, contrary to the usual observation, there was in some instances a slight increase in respiration during the 2nd hour, as for example in Experiments 10 and 15, though in no case did such an increase occur in the control tumors. This phenomenon cannot be explained unless it is assumed that dormant parts of the tumor tissue recovered when brought back to normal conditions. No marked differences in the water content of the experimental and control tumors can be seen. The figures in each group vary between 79 and 81 per cent. These are the usual figures for this type of tumor, as determined by various investigators and by my own observations (4). The water content of the tumor thus remained unchanged by refrigeration despite a loss of about 10 per cent in body weight.

The respiratory quotient was found to vary from 0.7 to 0.9 in the tumors of both the experimental and control group. The average values of oxygen uptake, respiratory quotient, and glycolysis thus showed no appreciable difference in the 2 groups. The average figures for oxygen consumption, slightly higher for the control tumors in the 1st hour of respiration, 0.344 as compared to 0.260, approached each other more closely in the 2nd hour, being in both cases between 0.297 and 0.269. This is in agreement with the observations made in several refrigeration experiments where a slight increase in oxygen consumption in the 2nd hour of respiration was noted. The average figures for lactic acid production of both experimental and control tumors lay in the same range, serving as another evidence of the failure of refrigeration to produce any appreciable effect on the metabolic rate.

The oxygen uptake and respiratory quotients were determined also for excised kidneys of mice kept at the lower temperatures and for controls, in order to determine whether a difference in susceptibility toward refrigeration exists between normal and malignant tissues. Kidney tissue was selected, since it had been shown in previous experiments (5-7) to have the most uniform respiration. The technic already described was used and the results were calculated on wet weight of the kidney tissue. The water content of the kidneys from both experimental and control mice varied in the range between 76 and 78 per cent. Observation as to oxygen uptake and respiratory quotient for 6 ex-

perimental and 6 control mice (Table III) showed no marked deviations. The oxygen consumption during the 1st and 2nd hours of respiration as well as the respiratory quotient, varied within the same range in both groups of animals. The same can be said of the average figures of all 6 experiments.

Summary and Interpretation of Results

Ninety-five mice weighing between 25 and 30 gm. were exposed to environmental temperatures of 5 to 7°C., either intermittently or continuously for from 8 to 48 hours. For mice of this size, this temperature and this period of time represented the limit of endurance. A high mortality rate occurred among mice during the 1st period of treatment. Only those tumor-bearing mice which survived the longest periods of treatment are considered in this paper.

In regard to the observations made by Bischoff and Long (1), it does not seem justifiable to compare these with the results recorded here, although they are similar to a certain extent. These authors kept their mice at temperatures from −2 to −5°C. for short periods and later transferred them to a temperature of 15°C. while our animals remained at temperatures from 5 to 7°C. continuously up to 48 hours. The disappearance of some of the tumors in our experiments, while Bischoff and Long obtained no lasting effect on tumor growth, may possibly be explained by the hypothesis that subcritical low temperatures over a longer period of exposure exert in some cases a more harmful effect on the animal as a whole than lower temperatures for shorter periods. This assumption is supported by the observation that, since no significant changes in the viability of the malignant

Table III: Oxygen Consumption and Respiratory Quotients of Excised Mouse Kidneys in Vitro in 0.2 Per Cent Dextrose Ringer-Phosphate Solution Buffered to pH 7.4 at 37.5° C. from Refrigerated and Control Mice *

	Refrigerated kidneys *in vivo*				Control kidneys of normal mice		
No. of experiment	QO_2 in 1st hour	QO_2 in 2nd hour	R. Q.	Remarks	QO_2 in 1st hour	QO_2 in 2nd hour	R. Q.
1	3.05	2.97	0.89	Mouse kept at 5° C. for 18 hours	3.36	2.85	0.91
2	2.79	2.15	0.86	Mouse kept at 5° C. for 24 hours	3.00	2.88	0.92
3	2.36	2.40	0.82	Mouse kept at 6° C. for 36 hours	2.30	2.18	0.89
4	2.98	2.24	0.75	Mouse kept at 5° C. for 48 hours	2.96	2.78	0.93
5	2.20	2.1	0.81	Mouse kept at 5° C. for 48 hours	2.58	2.17	0.83
6	2.89	3.01	1.85	Mouse kept at 5° C. for 48 hours	3.22	3.00	0.88
Average	2.71	2.46	0.83		2.90	2.64	0.89

* For each experiment 14 vessels were used and the average results calculated on wet weight are recorded.

Tumors disappeared completely in 5 mice; in others a small decrease or an arrest of tumor growth occurred during refrigeration. The arrest of tumor growth in most instances was only temporary, for the tumors increased in size when the animals were returned to normal conditions. Tumors of larger size, 16 to 18 days after inoculation, either did not change in size or ulcerated during refrigeration. The evaluation of the effect on proliferation was based on comparisons with tumors grown in control mice under usual conditions. The increase in size of this type of tumor is usually 0.1-0.2 cm. in diameter per day during the first 2 weeks after measurable dimensions have been attained. No appreciable effect of low environmental temperatures on the respiration and aerobic glycolysis of excised tumor or on the kidneys could be detected.

Since the viability of the neoplastic cells remained unaffected, as shown by the metabolic rate, disappearance of the tumors can scarcely be attributed to the effect of the low temperatures. It may have been due to the impairment of the general health of the animals exposed to such temperatures. This assumption is justified by the observation, frequently made, that tumors disappear when the host contracts another disease.

cells were produced by refrigeration under these conditions, the disappearance of the few tumors is probably an indirect effect of impairment of the general condition of the host rather than a direct effect on the neoplasm itself.

In conclusion it may be said that observations made in these experiments; *i.e.,* the high mortality rate, the comparatively few successful results, and the unaffected viability of the tumor cells, indicate the inadequacy of refrigeration in the treatment of malignant growth.

My sincere thanks are given to Warner, Schering, and Glatz, Inc., for financial aid; to Mr. F. Sellmer, Associate in the Physical Laboratory of the Department of Hospitals, for constructing the double razor; and to the Works Progress Administration for a technical helper assigned to me for these experiments.

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The Effect of Age Upon the Connective Tissue of the Uterus, Cervix, and Vagina of the Rat*

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Recently Loeb, Suntzeff, and Burns (7, 8) reported that advancing age in the mouse is accompanied by changes in the stroma of the uterus, cervix, and vagina. The most significant of these is an increase in the amount of fibrillar and hyaline connective tissue, which is first manifest in the earliest months of life, progressing only slightly throughout the sexually active period, but becoming pronounced after the age of 18 months. In studying the effects of age and endocrine factors upon certain tissues in the rat, we have found, in general, changes similar to those described by Loeb and his associates in the mouse. In addition, evidence was obtained which indicates that the reproductive history of the animal plays an important role in the determination of the amount of connective tissue deposited in the genital organs and, furthermore, that this effect may vary somewhat in different strains of rats.

Materials and Methods

Two hundred and twenty rats were sacrificed at ages ranging from 30 to over 800 days. Approximately half of these were from the Albany (A-S) strain, which is characterized by low fertility and a fairly high incidence of spontaneous fibroadenomas (3). The remaining half were from the Vanderbilt (V-S) strain, in which tumor incidence is low and fertility high (4). No animals with tumors were used for these particular studies. Up to 1 year of age, all rats were virgins. The groups of animals over 1 year of age consisted of breeding females and nonbreeders which were virgins of either strain, or sterile females of the A-S strain (12).

The reproductive tract was fixed in Bouin's fluid. Representative sections were taken from the uterus and vagina of each rat, and complete serial sections of the cervices of 54 rats were cut. Sections from all tissues were stained routinely with hematoxylin and eosin. In addition, Goldner's modification (6) of the Masson trichrome method was used for staining the connective tissue. By this technic both collagen and reticulum are stained green, smooth muscle and epithelium red or orange-red, and nuclei brownish-black to reddish-brown. The method does not differentiate collagen from reticulum, and in this paper, therefore, no effort will be made to separate the two. In the descriptions to follow, the term collagen is employed in a collective sense for both reticular and collagenous tissue.

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Changes in the Uterus with Advancing Age

Alterations in the endometrium.—The entire endometrium of the immature animal (30-40 days) in both strains is strikingly cellular. The stromal cells appear round or oval, with scant cytoplasm, which can only infrequently be observed, and large round or oval nuclei which have clearly defined nuclear membranes, prominent nucleoli, and chromatin scattered in coarse aggregates. There is a greater abundance of cells in the inner portion of the endometrium than in the outer region near the muscle layer.

A fibrillar meshwork lies between the stromal cells. Immediately beneath the epithelium which lines the uterine lumen, the fibrils are delicate, separate, and lightly staining, but deeper in the endometrium they become thicker, more dense, and stain more deeply. This is especially true in the outermost portion, near the myometrium, where the cells are relatively less abundant and the fibrillar connective tissue is more conspicuous than elsewhere.

From the immature to the 3-month stage there is a noticeable increase in collagenous tissue in the endometrium. Immediately beneath the lining cells the fibrils remain comparatively thin. More deeply, however, they have a tendency to become aggregated into fiber bundles. This process is most pronounced in the neighborhood of glands, about which the fibers are arranged in concentric rings, and in the outermost portion of the endometrium, where the individual fibers

are thicker and the fibrils which compose them are more dense than elsewhere.

The stromal cells are generally more abundant in the subepithelial portion of the endometrium and become progressively less numerous as the myometrium is approached. Beneath the lining epithelium they still resemble those seen in the younger animals, but deeper down they become somewhat elongated, probably from compression by the denser collagenous fibers; the cell nuclei in most instances, however, remain quite large and vesicular.

In the period from 3 to 6 months there is a steady but highly variable further increase in the deposition of collagen in the endometrium, which differs regionally in its cellular composition and structure. While the cells directly under the lining epithelium usually retain their round vesicular nature and lie in a loose and relatively fine fibrillar network, the extent of this type of tissue varies in different animals; in some all or part of this superficial endometrium is composed of a dense meshwork of thickened fibrils in which are embedded fusiform cells. In the middle portion of the endometrium, where the glands are situated, the connective tissue may be composed of a dense meshwork of coarse fibers between which lie stromal cells with round or oval vesicular nuclei. Sometimes the collagenous fibers are arranged in whorl-like laminations around the glands, compressing the cells between them. Sometimes they appear to form a homogeneous sheet of tissue which, under high magnification, is found to be a closely knit network of extremely fine fibrils (Fig. 1). In these areas the cells have no discernible cytoplasm, but do have characteristic hypertrophied, vesicular nuclei containing scant chromatin, prominent nucleoli, and indistinct nuclear membranes (Fig. 1). In the outermost region of the endometrium the collagen usually becomes much more dense and consists of coarse undulating fibers which interlace or run in parallel bundles and compress the stromal cells which lie between them. These cells are for the most part oval or spindle-shaped and have elongated nuclei, which are usually vesicular, although they may often appear shrunken and pyknotic.

From the age of 6 months on, there is an intensification in the formation of endometrial collagen. The rate of production, the amount laid down, and the manner in which it is arranged depend, apparently, not only on the age of the animal but also upon its reproductive history. In the virgins of both strains, and in A-S sterile females, there is, as age advances, a relatively rapid increase in the deposition of collagenous tissue (Fig. 2). In old virgin rats, particularly those of 18 months or over, the most superficial endometrial connective tissue, which in younger animals is loose and finely fibrillar, containing an abundance of characteristic cells with large vesicular nuclei, is replaced by denser collagenous tissue. Although there is considerable variability in the extent of this replacement and in the abundance of the stromal cells, the tendency for the condensation of collagen in this region is unmistakable.

The middle portion of the endometrium is not uniform in character. In some situations this is composed of coarse, deeply staining fibers which are wrapped around the glands like sleeves, compressing the cells lying between them. In intervening areas the character varies from well stained, definitely fibrillar tissue to lightly stained islands which only on very high magnification are seen to be composed of an extremely fine fibrillar meshwork. Indeed, not infrequently a considerable portion of this part of the endometrium is composed of sheets of the lightly staining, delicately fibrillar collagenous tissue, the cells of which, as in the younger group, are hypertrophied and contain relatively large, pale nuclei with scant chromatin and prominent nucleoli (See Fig. 1).

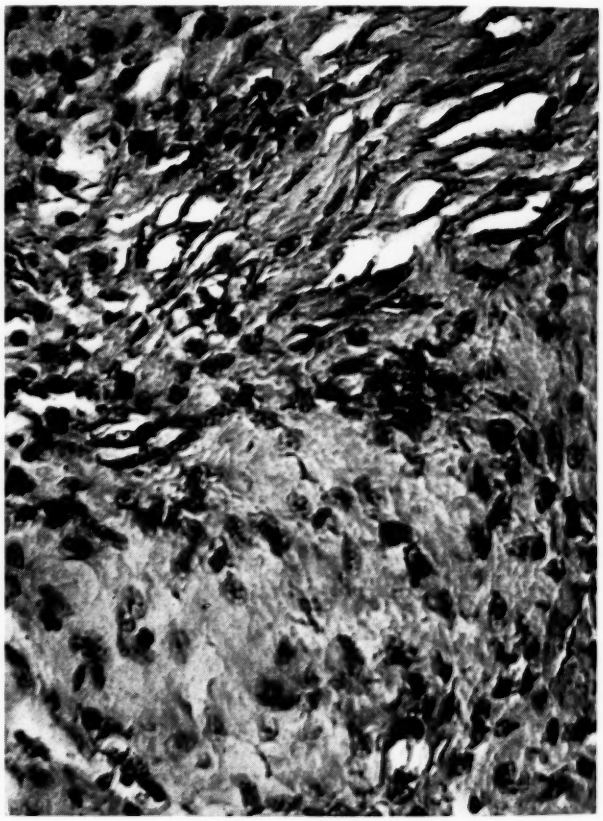

FIG. 1.—Section from uterus of an A-S rat 6 months old, showing regions of finely fibrillar collagen containing hypertrophied cells with prominent nucleoli and indistinct nuclear membranes. Mag. × 330.

Finally, in the deepest portion of the endometrium the connective tissue consists of coarse, deeply staining fibers. With advancing age, particularly after 18 months, there is a tendency for the collagenous tissue

to become increasingly condensed into thick fibers or broad bands (Fig. 4). Sometimes the central portions of the wide bands appear homogeneous or hyaline and may display an alteration in staining properties, showing, in sections stained by the modified Masson technic, a greater affinity for the red instead of the green dye (Fig. 6). The fiber bundles are usually closely packed together and in most instances compress the stromal cells lying between them.

FIG. 2.—Cross section of uterus of 1 year old V-S virgin rat. Mag. × 28. Compare with Fig. 3 for size.

In fertile breeding animals of both strains the rate of collagen deposition in the endometrium is slower than in females which have never borne young. In the breeding V-S rats, which were uniformly very fertile, the uterus remained fairly small in diameter as compared with the size in virgins of the same age (Figs. 2 and 3). The endometrium was characterized by a much greater compactness of the fibrillar meshwork as a whole and by the presence of dense collagenous tissue in the inner portion instead of the loose, delicate network commonly seen in the younger animals. In most instances the entire endometrium was remarkably cellular, and frequently the majority of the cells had nuclei of the vesicular type.

In about one-half of the animals comprising the V-S breeding group over 18 months of age, the endometrial stroma appeared to be composed of dense, almost hyaline collagen. Careful examination with the oil immersion lens, however, revealed that this consisted actually of a sheet of very fine fibrils, although it was extremely difficult, if not impossible, always to discern the fibrillar architecture. Frequently—and this was particularly true in the peripheral portion of the endometrium—the collagen was demarcated into broad interconnecting bands by the stromal cells.

In the A-S breeding animals there was less uniformity in the size of the uterus. In a number of instances this was of large diameter and contained a

FIG. 3.—Cross section of uterus of 1 year old V-S breeding rat. Mag. × 28. Compare with Fig. 2 for size.

generous amount of connective tissue, thus resembling those of virgin rats. In seeking an explanation for these observations, examination was made of the reproductive histories, and also of vaginal smear records when such were available. It was noted that the animals with large uteri had not been pregnant more than once or twice, usually because of an ovarian dysfunction which manifested itself by a vaginal hyperestrus (12). On the other hand, those which had given birth to from 5 to 7 litters had small uteri, similar to those of V-S animals whose reproductive capacity was about the same. These observations tended to confirm the general impression that the deposition of large amounts of connective tissue is associated with the nonpregnant state.

To check on the observations further, the thickness of the endometrium and the myometrium of breeding and nonbreeding animals of both strains was measured. The results are summarized in Table I. The findings must be regarded as only suggestive, since

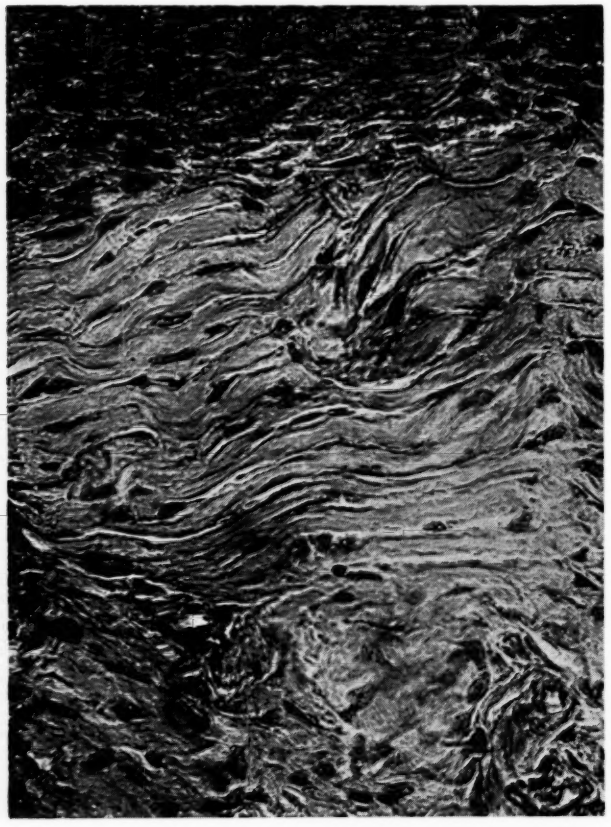

FIG. 4.—Section from uterus of an A-S rat 17 months old, showing wide bands of collagen in the outermost portion of the endometrium. Mag. × 210.

endometrium, the measurements of the myometrium show no significant variations among the groups.

Alterations in the myometrium.—Equally striking changes occur in the myometrium with advancing age. Frequently in breeding rats the condition of the muscular coats suggests the age of the animal when it is impossible to assign the uterus to any particular age group on the basis of endometrial changes. Up to the age of 6 months there is no significant increase in the interstitial tissue of the myometrium, and only infrequently is there found a noticeable increase in connective tissue surrounding the larger arteries present between the 2 muscular coats. As age advances, however, the myometrial stroma becomes progressively more abundant, and in the oldest age group (1.5 to 2 years) constitutes a considerable portion of the muscular wall.

Invariably the earliest changes are noted in the inner or circular smooth muscle. In the large uteri the muscle cells in this region often appear atrophied, and the muscular ring may give the impression of being stretched, as if distended by the pressure of the enlarging endometrium. In virgins and breeders of both strains the circular muscle layer becomes in most instances extensively invaded by connective tissue before significant alterations are observed in the outer longitudinal muscle coat (Fig. 5). In old rats, however, the collagenous bands which surround the muscle bundles of the longitudinal layer are decidedly thicker,

TABLE I: SUMMARY OF QUANTITATIVE DATA GIVING THE AVERAGE WIDTH, IN MILLIMETERS, OF THE ENDOMETRIUM AND MYOMETRIUM OF THE VARIOUS GROUPS

Groups	No. of animals	Age (months)	Endometrium Mean ± P. E. mm.	Endometrium Significance ratio [5]	Myometrium Mean ± P. E. mm.
V-S virgins	14	12 [3]	1.72 ± 0.07	8.7	0.80 ± 0.02
V-S breeders	15	12	0.97 ± 0.05	..	0.81 ± 0.05
V-S breeders [1]	18	19 [4]	1.10 ± 0.05	3.0	0.78 ± 0.04
A-S breeders [2]	20	19	1.36 ± 0.07	3.7	0.74 ± 0.03
A-S virgins	16	19	1.81 ± 0.10	..	0.70 ± 0.03

[1] The average number of litters was 5 to 6.
[2] The average number of litters was 3 to 4.
[3] In this and the following group, each animal was 12 months old.
[4] In this and the following 2 groups, the ages ranged from 14 months to 2 years, the average being 19 months.
[5] The significance ratio is the ratio of the difference between the means to the probable error of the difference. If it is 3 or over, the data are considered reliable.

the number of animals in each series is small, but the results confirm the impression obtained from histologic examination. Comparison of 1 year old V-S virgins and V-S breeders shows that the mean width of the endometrium in the virgins is significantly greater than that of the breeders. In groups in which the mean age is 19 months, the average width of the endometrium of the A-S breeders exceeds that of the V-S breeders, but is in turn exceeded to an even greater degree in the A-S females which had never borne young. In contrast to the differences noted in the and the fibrous trabeculae which dip into the myometrium from the serosa become increasingly prominent. In extreme cases the overgrowth of the collagen reduces the muscle bundles to insignificant proportions.

The increase in perivascular connective tissue which forms in sleeve-like fashion around the blood vessels in the myometrium is clearly demonstrated in animals which have reached 1 year of age, and it becomes more pronounced in progressively older females. Sometimes this connective tissue is composed of well-formed, fairly thick fibers which not only surround

the arteries but also produce a continuous wide ring of collagenous tissue between the 2 muscular coats. In most cases, however, this connective tissue is arranged more irregularly and actually replaces muscle cells in both muscular layers (Fig. 6). It consists of fairly fine interlacing fibers or of sheets of pale staining hyaline collagen.

Changes in the Cervix with Advancing Age

As we have not been able to find any satisfactory description of the cervix of the rat, it may be pertinent to mention its main features here. The rat cervix may be said to begin at the point where the uterine horns fuse. It contains 2 canals which are prolongations of the uterine lumina. Each canal communicates independently with the vagina (Fig. 7). In approximately the upper third of the cervix, which represents the uterine portion (Fig. 7, **a**), the epithelium lining the cavities is columnar in type, but at the point where the obliquely directed lumina become the relatively narrow, straight cervical canals (Fig. 7, **b**), there is an abrupt change of epithelium to the vaginal or stratified variety. It is at this level (Fig. 7, **b**) also that the outer longitudinal uterine muscular coats diminish in width rapidly and disappear in the loose connective tissue surrounding the cervix. The circular smooth muscle of the uterine horns is continued into the cervix, and in the vertical portion of the organ (Fig. 7, **c**), consists chiefly of circular bundles. These are especially prominent in the upper half of the vertical cervix (Fig. 7, **c**), but in the distal portion they begin to be diluted by an increased amount of connective tissue which forms the chief bulk of the cervical lips (Fig. 7, **e**). Throughout the length of the cervical canals an inner longitudinal muscle coat is developed on either side. On the medial aspect these longitudinal muscle layers flank a central core of circular muscle bundles, with which they merge obliquely at the tip. On the lateral sides they are continued into the lips, mixing with the circular muscle present and extending into the vagina to form its longitudinal muscle coat.

The stroma of the rat cervix at all ages, but especially in the young, is quite cellular. In its uterine portion

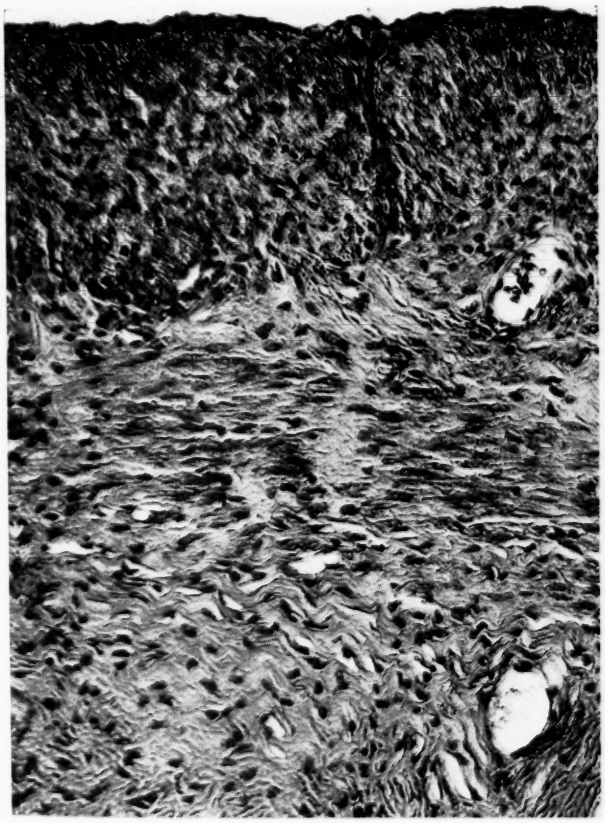

Fig. 5.—Section from uterus of an A-S rat 18 months old, showing extensive invasion by connective tissue of the inner or circular muscle coat in contrast with insignificant alterations in the outer or longitudinal layer. Mag. × 200.

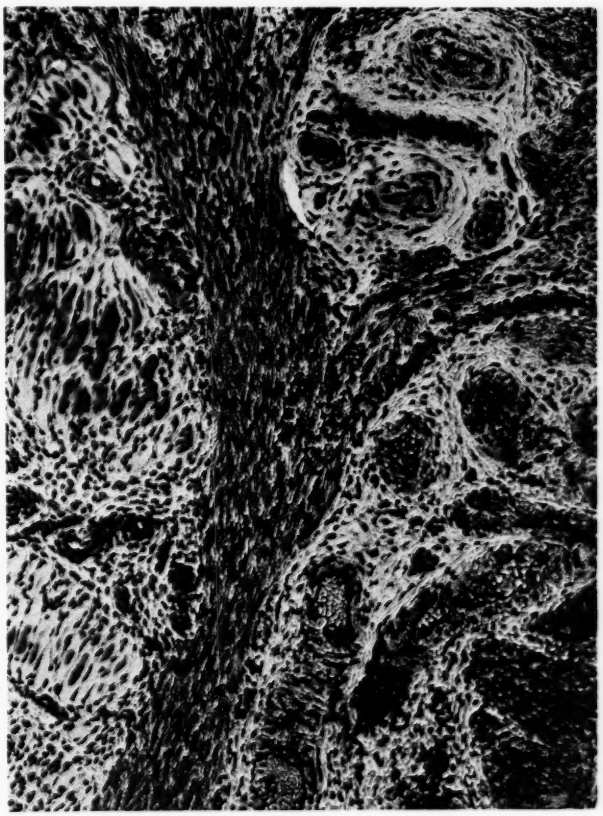

Fig. 6.—Section from the uterus of a V-S rat 19 months old, showing the increase of perivascular connective tissue and wide collagen bands in the endometrium. The dark central regions have taken the red (fuchsin) stain instead of the usual light green dye, here taken up only by the peripheral portions of the bands. Masson trichrome stain. Mag. × 130.

the connective tissue beneath the epithelium lining the canals, like that of the superficial endometrial stroma, is delicate and fibrillar and persists in this form for variable distances beyond the point where the uterine (columnar) type of lining epithelium gives way to the vaginal (stratified) form. It then changes gradually, becoming a dense matrix of fibers, which run parallel to the longitudinal muscle layer and crowd the cells lying between them. The connective tissue surrounding the bundles of circular muscle consists of irregularly arranged collagenous fibers which become

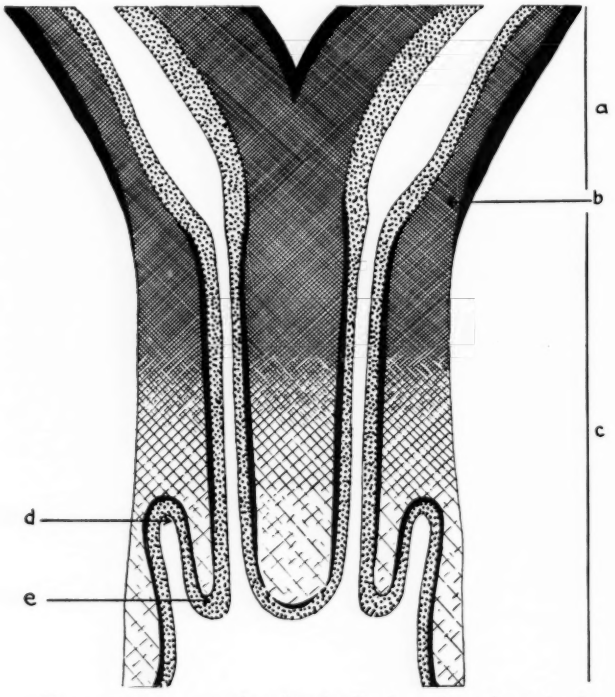

FIG. 7.—Diagram of a longitudinal section through the typical cervix of the uterus of the rat. Longitudinal smooth muscle is represented in solid black; circular smooth muscle is cross-hatched; subepithelial tissue is stippled; epithelium is not shown.

a: uterine portion of the cervix; **b**: level at which columnar epithelium changes to a stratified epithelium, and the uterine longitudinal smooth muscle becomes lost in the surrounding pelvic fascia; **c**: vertical portion of the cervix; **d**: vaginal fold; **e**: lateral lip of the cervical canal.

increasingly dense and more conspicuous in the region of the outer cervical lips, where the muscle bundles become sparse. Under the epithelium of the lateral walls of the cervical lips the pattern most frequently observed is that of fairly thick fibers which interlace freely; it then changes either gradually or abruptly to the finely fibrillar meshwork that usually characterizes the vaginal folds with which the cervix becomes continuous.

In the cervix, as in the uterus, there is a considerable increase in collagenous tissue from the immature to the 3-month stage, resulting in enlargement in all three dimensions. As age advances the collagenous tissue increases in amount and density, the most striking acceleration occurring in those animals which do not breed. Thus, discounting the usual variations of biological material, the cervices of 1 year old virgins are in the main decidedly larger than those of breeders of the same age (Figs. 8 and 9). Indeed, the outer lips of the cervical canals may become so enlarged and thickened that they project into the vagina, not infrequently converting the normally separate openings of the 2 canals into a common outlet (Fig. 8).

Changes in the Vagina with Advancing Age

The connective tissue even in the immature animals is usually a closely interwoven collagenous meshwork in which are embedded a considerable number of fibroblasts containing large vesicular nuclei. Occasionally it is difficult to make out any fibrils in this meshwork, but where the section chances to be thin the fibrillar character is quite clear. As age progresses the connective tissue becomes more dense. In some regions there is a tendency for the collagen to become aggregated into coarse, closely packed fibers. This is observed most often in the anterior wall near the urethra. In other areas, the connective tissue may resemble a dense, almost hyaline sheet in which the fibrillar structure cannot always be made out even with high magnification. The proportions of the coarse, deeply staining fibrous tissue and the very finely woven, lightly staining, fibrillar type vary widely not only in different animals but also in different regions of the vagina in the same animal. While the connective tissue near the urethra is usually coarse, that which is present in the vaginal folds is of the finely fibrillar or hyaline-appearing variety. The differences in the number and nature of the fibroblasts characterize for the most part the young and old vaginae. In the young the cells are abundant and large, with typical vesicular nuclei. In the old they are relatively few in proportion to the amount of collagenous tissue, and appear predominantly flattened and shrunken. Between these two extremes wide variations in cellular composition exist. In some 1 year old females, for example, both breeders and virgins, the stroma may contain relatively few cells, whereas in much older animals a remarkable cellularity may be observed.

Abnormal Staining Reactions by the Trichrome Stain

In some of our sections prepared by Goldner's modification of the Masson trichrome stain (6), as well as in sections stained by Masson's original technic (9), we have encountered unusual staining reactions in connective tissues. Whether or not these reactions are significant and result from actual biochemical modi-

fications of the tissue, or whether they are artifacts, we do not know. To date, the results of our investigations as to the cause of the phenomenon are confusing, and the final solution is not yet at hand. Brief mention will be made, however, of our observations in order to call attention to the difficulties which may accompany the use of this technic for the study of connective tissue in the female reproductive system, at least of the rat.

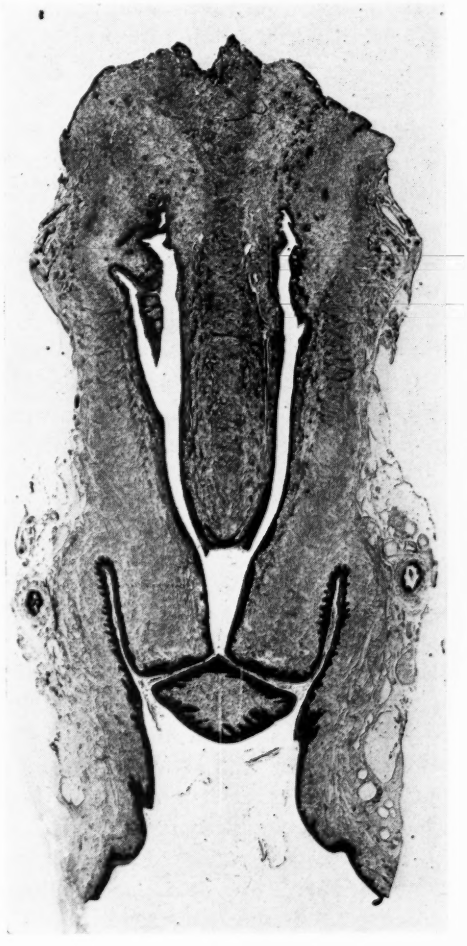

FIG. 8.—Longitudinal section through the cervix of a 1 year old virgin V-S rat, showing the projection of the large lateral lips into the vagina, thus creating a common passage for the 2 cervical canals. Mag. × 8. Compare with Fig. 9 for size.

The unusual finding is that in places the connective tissue, instead of being stained by the light green dye, as is to be expected, is stained red by the fuchsin. If a connective tissue fiber is fairly broad, the central portion may be red while the peripheral portion is green (Fig. 6). Sometimes almost the entire fiber may stain green but with red tinges in its course. If the stroma is finely fibrillar, as so frequently occurs in the vagina, many of the fine fibrils in irregular areas appear red and fade gently into others which are green. In the vagina the change occurs most frequently under and parallel to the epithelium but not immediately adjacent to it. Other sections, from different animals or from different regions of the same organ, stained in the same dish with sections which give the peculiar staining reaction, fail to show the unusual affinity for fuchsin. Studies of serial sections and modifications of procedure have thus far not yielded clarifying results. Whatever may be the basis for this tinctorial abnormality, it is apparently not related to the age or sexual activity of the animal, for it is observed in immature animals as well as in the old and at all phases of the estrous cycle in sexually mature rats.

FIG. 9.—Longitudinal section through the cervix of a 1 year old breeding V-S rat, showing the 2 cervical canals communicating separately with the vagina. Mag. × 8. Compare with Fig. 8 for size.

DISCUSSION

These studies in general confirm those of Loeb and his associates with respect to the increase in the amount of collagen and in the density of the collagen fibers with advancing age. There are, however, several minor points of difference between their observations in the mouse and ours in the rat, which suggest species variations. Whereas they found only "a gradual slight increase in the relative amount of connective-tissue fibrillae from the age of three weeks to the age of four months" (8), we have been impressed with the striking increase in the rate of connective tissue deposition which occurs between the ages of approximately 30 days and 3 or 4 months. In the mouse the increase in collagenous tissue "appears to make little advance throughout the sexually active interval, but it progresses again during the later period of life" (8), while in the rat the deposition of collagenous tissue appears to continue throughout the sexual

period and is influenced by the sexual history. Thus it was noted with striking frequency that the uteri and cervices of virgins were manifestly larger than those of breeding females of comparable age. This cannot be attributed to any direct relation of the size of individual organs to general body weight, for the body weights of virgin animals are on the average distinctly lower than those of breeding females (2, 5, 13).

In a previous study of the estrous cycles of rats in the A-S and V-S strains (12) it was observed that in the virgins of both strains vaginal smears containing only epithelial or cornified cells, or a mixture of these two, occurred during approximately 45 per cent of the almost lifelong observation period. In contrast, such smears were found in the V-S breeding animals for only about 13 per cent of the time, and in the A-S breeding rats for approximately 30 per cent. The increased proportion of the observation period during which the A-S breeding animals showed smears characterized by epithelial and cornified cells as compared with the V-S rats was due to the generally poor reproductive abilities of the former.

In the virgin rats the presence of epithelial and cornified cells can safely be taken as an index of estrogen activity, and the large amounts of collagenous tissue found in the accessory reproductive organs of these animals can probably be associated with the long-continued action of estrogen. These observations strongly suggest that estrogen stimulates the formation of collagenous tissue in these organs. This view is supported by the findings of Suntzeff and his associates (10), who reported that in mice receiving injections of estrogen a homogeneous hyaline substance having the staining properties of collagen was deposited in the uterus, cervix, and vagina. By using a method which differentiates collagen from reticulum, we have found that, as age advances, there is a transformation of the latter into the former in the endometrium (11). In preliminary studies of the effect of estrogen on the reproductive organs of the immature rat (unpublished data) a considerable increase in collagenous tissue, with a concomitant decrease in reticulum, has been observed after 2 weeks of intensive administration of estrogenic substance. All these findings point to estrogen as the determining factor in the formation of connective tissue in these organs.

The factors which induce the more restricted deposition of collagen in the breeding animals are not clear. This restriction, associated with the more limited period during which such animals have only epithelial or cornified cells in the vaginal smears, would superficially appear to be due to the decreased time during which estrogen acts on the accessory reproductive organs. It is known, however, that estrogens are abundant in pregnancy in certain species, and it is quite possible that this is true in the rat as well. If so, it seems probable that the action of estrogen on the connective tissue of the accessory reproductive organs is held in abeyance by some factor produced during pregnancy, probably progestin. This is considered likely, since progestin is known to restrict the action of estrogen on the vaginal mucosa (1).

In the V-S strain there were highly significant differences in width of the endometrium in virgins and fertile breeders of the same age. In the A-S animals, the differences between groups of breeders and virgins in which the average ages were comparable were not great but were nevertheless significant. Examination whenever possible of both the reproductive histories and vaginal smear records of the animals which were used for the histologic studies revealed that the A-S females which had given birth to from 5 to 7 litters usually had relatively small uteri. On the other hand, the animals with large uteri had had only 1 or 2 pregnancies and long periods of predominantly epithelial smears, indicative of a prolonged estrogenic activity. This is interpreted as supporting the concept that the proliferation of collagenous tissue is influenced by estrogen, and that the amount deposited is related to the length of time and extent of estrogenic stimulation, at least in the nonpregnant state.

Summary

In studies concerning the effects of age and endocrine factors upon certain of the connective tissues of the body, the uteri, cervices, and vaginae of rats, sacrificed at ages ranging from 30 to over 800 days, were examined histologically. Two strains of rats were employed, the (A-S) strain, characterized by low fertility and a fairly high incidence of spontaneous mammary fibroadenomas, and the (V-S) strain, in which fertility is high and spontaneous development of tumors is low.

It was found, in general, that as age advanced there was an increase in the amount and condensation of the collagenous tissue in the uterus, cervix, and vagina of the normal rat. In the endometrium this increase was observed to be greatest from the immature to the 3-month stage. From then on there was great variability in the further deposition of collagen. Furthermore, the rate of formation, the amount deposited, and the manner in which the collagen was arranged depended, apparently, not only on age but also upon the sexual history of the animal. In virgins the growth of connective tissue was greater than in breeding animals and resulted in the frequent development of strikingly larger uteri and cervices than were usually found in highly fertile breeders of comparable age. The observations in general tended to associate the deposition of collagenous tissue in the accessory reproductive organs of the rat with the long-continued, unmodified influence of estrogen in the nonpregnant state.

Beginning with approximately 1 year of age there was a progressive increase in the amount of connective tissue in the muscle layers of the uterus. The earliest changes were invariably observed in the inner or circular smooth muscle coat, although in old rats significant replacement was also frequently seen in the outer or longitudinal layer. Large amounts of collagenous tissue also developed around the arteries, especially between the muscle coats.

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Structure and Origin of Uterine and Extragenital Fibroids Induced Experimentally in the Guinea Pig by Prolonged Administration of Estrogens*

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The purpose of this communication is to present the findings of a detailed microscopical study of the sites of origin and stages of development of the subserous fibroid tumors induced in guinea pigs by prolonged administration of estrogens. Details of treatment of the animals are given in the explanations of Plates 1-5.

Subserous uterine tumors which can be induced in guinea pigs by prolonged administration of estrogens, as described by Nelson (26, 27), were found to be fibroids. Lipschütz, Iglesias, and Vargas (13, 18, 22) have shown that extragenital tumors in the abdominal cavity, induced by estrogens, also were fibroids. The localization of these tumors at various sites on the uterus, pancreas, kidney, spleen, etc., have been described by Iglesias (5), Vargas and Lipschütz (32), Bellolio (1), Murillo (25), Rodríguez (30), and Lipschütz, Iglesias, and Vargas (15). Several examples of uterine and extragenital fibroid tumors, induced in guinea pigs treated with estradiol monobenzoate, are shown in Plate 1. We have demonstrated that similar fibroids at these sites can be induced by administration of all natural estrogens (estradiol, estrone, estriol) free or esterified (dipropionate, caprylate, benzoate-butyrate). Similar results were obtained by the administration of such artificial estrogens as stilbestrol, in the free form, by Lipschütz and Vargas (19), or esterified, by Lipschütz, Vargas, and Bruzzone (21) or by hexestrol, by Lipschütz and Egana (12).

General Characteristics of Fibroids

The central portion of a typical large apical uterine fibroid is composed predominantly of fibrous tissue with few cells scattered between the bundles of fibers. In some areas bundles of smooth muscles may be found; in others the loose fibrous tissue may be edematous. These characteristics are shown in Plate 2, Figs. 1-A, 1-B, and 1-C.

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These experimentally induced abdominal tumors present a smooth surface formed of a capsule composed of flattened superficial cells (Plate 2, Figs. 2-A and 2-B). The cells beneath the capsule resemble fibroblasts. These cells have definite boundaries or they are separated from each other by collagenous fibers (Plate 4, Fig. 11-C).

The masses of fibroid tumors arising from the apex of the uterine horn may enclose the tubes or large tubal cysts. The demarcation between the muscular coat of the tube and the tumor is not always sharp. In some instances, especially when the apical fibroid is small, the tumor is in close contact with an abundance of smooth muscle and adipose tissue (Plate 2, Fig. 2-B), whereas the fibroid itself, to whose area the muscle tissue belongs, is covered with a capsule (Plate 2, Fig. 2-A). In some places fibrous tissue may also be in direct contact with adipose tissue (Plate 4, Fig. 11-A). Small epithelial tubes, probably belonging to the Wolffian body (Plate 2, Fig. 2-B) may be found surrounded by muscle tissue or connective tissue, as previously described by Lipschütz (10). These features indicate that the apical fibroid is a tumor of the mesosalpinx. In the untreated normal animal patches of compact smooth muscle may also be found near the tube. These masses of muscle probably hypertrophy under the influence of the estrogen, and are found subsequently in contact with, or incorporated in, the growing fibroid.

Apical uterine fibroids occurred only in castrated females treated with estrogens. On the other hand fibroids occurred at other sites in the abdominal cavity in both normal and castrated guinea pigs.

Abdominal fibroids can be induced experimentally by estrogens both in noncastrated and castrated males. But larger quantities of estrogens administered over longer periods of time are required to bring about these tumors in males than in females. The histological structure of the fibroids is the same in the males as in the females. Nevertheless, there is a pronounced sex specificity in the collagenous tumoral reaction, as described by Koref, Lipschütz, and Vargas (7), Jed-

licky, Lipschütz, and Vargas (6), Chaume (17), Szabó (31), and Palma (28).

In the castrated male a fibroid tumor is formed also at the site of ligature of the spermatic cord. These inguinal tumors, like the apical uterine tumors, may be rich in smooth muscle.

As Lipschütz (11) has pointed out the structure of the experimentally induced fibroid tumor of the male or female guinea pig differs considerably from the spontaneous fibromyoma in women. In the experimental fibromyoma there is much less order, particularly in the arrangement of the fibrous tissue. The compact myomatous tissue shows an orderly arrangement in both types of tumor. Another important difference is the abundance of fibroblasts near the capsule in the experimentally induced tumor (Plate 2, Fig. 2-A; Plate 4, Figs. 11-B and 11-C). In addition numerous cells of other types, some with pyknotic nuclei, may be found in these fibroids (Plate 2, Fig. 3).

Small nodules or excrescences, called "tumoral seed" (Plate 2, Figs. 4-A and 4-B) deserve special attention. These have been found by Vargas and Lipschütz (32) on the inner abdominal wall, the surface of the stomach, the spleen, and elsewhere. Their structure is similar to, though not always identical with, the typical experimental fibroid. Cells rich in cytoplasm may predominate in these small nodules (Plate 2, Fig. 5) and an intimate relation with the mesothelial cells of the serosa is often evident. The structure of some of these small nodules is the same as that of the large fibroids. The cells at the periphery have the shape of fibroblasts and there is an abundance of fibrous tissue in the center. In general these small excrescences seem to have a strong tendency to undergo sclerotization, as shown in Plate 3, Figs. 6-A and 6-B. Although it is probable that the small excrescences may develop into large fibroids it is not likely that every small nodule or tumor seed of this type is potentially a large fibroid. Apparently their development into large tumors is often interrupted by sclerotization.

Abundance of Cells

In our experimental animals there was often a remarkable augmentation of the mesothelium of the serosa, as shown in Plate 3, Figs 7-A and 7-B. Although the difference between the serosal cells of normal guinea pigs and guinea pigs under treatment with estrogens is not always conspicuous, nevertheless in some instances there is a striking accumulation of cells rich in cytoplasm on the surface of the tumor. The importance of these findings, however, should not be overestimated. A similar transformation of the mesothelium of the serosa may occur as a result of inflammation of the peritoneum. The findings raise the relevant question whether there is in these cases a tumoral proliferation of the mesothelium of the serosa and of the endothelium of vascular and lymphatic spaces, or of the cells of the mesenchyme which are in immediate contact with the endothelium.

In 1938 Lipschütz, Vargas, and Iglesias (22) described an example of apparent proliferation of the endothelium of vascular or lymphatic spaces in a fibroid of the abdominal wall. In the past 18 months many additional observations have been made. Typical examples are shown in a series of sections of small intramural fibroids of the uterus of a guinea pig treated for 3 months with estrone (Plate 3, Figs. 10-A to 10-F). The cleft in the muscular septum between the uterine horns (Fig. 10-A) is lined with endothelium and may be a lymphatic or vascular space. A fibroid is in process of formation in the myometrium (Fig. 10-B). Neoformation of tumoral tissue here appears to be intimately related to the underlying cells of the mesenchyme and not with the endothelium. The club of tumoral tissue consists at its base of dense fibrous tissue and at its periphery of spindle cells, easily distinguishable from the muscle fibers of the myometrium in which the fibroid is growing (Fig. 10-C). The cells of the small nodule shown in Figs. 10-D and 10-E are different from the spindle cells of Fig. 10-C. These cells have the appearance of undergoing necrosis. In Fig. 10-F definite evidence of necrosis is shown.

Another aspect of cellular proliferation is shown in Plate 4, Fig. 11-A, from a splenic tumor. At the top the tumor is infiltrating the pancreas. But the contact of the tumor with the pancreas is incomplete, so that a cleft remains between the two. There is abundant cellular proliferation in this cleft. These cells may be mistaken for proliferating endothelium; more probably they are the cells of the peripheral layer of the tumor, as shown also in Plate 4, Figs. 12-A and 14.

One may find also an accumulation of cells surrounded by adipose tissue, as in Plate 4, Fig. 13, from a tumor of the hilum of the spleen. These accumulations are rich in blood vessels, as shown in Plate 4, Fig. 12-B, from an apical tumor. The processes responsible for this accumulation of cells appear to be intimately related to the blood vessels. One may assume that the accumulation of cells around the blood vessels is due to proliferation of mesenchyme cells underlying the endothelium, but the question is far from being settled.

Infiltration

The extra-uterine fibroids invade certain tissues. The behavior is different according to the organ. A fibroid in close contact with the kidney or the spleen is always separated from these structures by a sharp line; *i.e.*, by the fibrous capsule of the organ which may be very

much thickened at the line of contact. On the other hand, other organs, such as the pancreas, liver, smooth muscle, and striated muscle are infiltrated by the tumoral tissue. There is a disintegration and destruction of lobules of the pancreas which are in contact with the tumor or are surrounded by it (Plate 5, Fig. 15). In the same way, though in a lesser degree, liver tissue suffers disintegration when in contact with the tumor (Plate 5, Fig. 16).

Infiltration of smooth muscle tissue has been studied in sections of the stomach and various parts of the intestine (Plate 5, Fig. 18). Intermingling of smooth muscle tissue of tumor and myometrium may occur also (Plate 5, Fig. 17). In general one may be in doubt as to how to interpret the condition; *i.e.*, whether there is only infiltration of the muscle tissue by the tumor or whether the muscle tissue participates itself in the proliferation. It is the same in the male with the vas deferens when surrounded by a tumor. The invasion and disintegration of striated muscle by tumors of the abdominal wall (Plate 5, Fig. 19) or of the diaphragm is also very remarkable.

The resistance of certain organs as kidney and spleen against invasion is all the more remarkable as their surface and hilum are so often the site of fibroids of considerable size.

General Fibrosis

Fibrous strands on the inner abdominal wall, in the mesentery, and other places often accompany fibroids or they are the only manifestation of the collagenous proliferative reaction as reported by Vargas and Lipschütz (32). These strands are very poor in cells. Lipschütz and Vargas (17) found accidentally also a fibrous reaction around the glands of Brunner in the duodenum. The thickening of the capsule of the kidney (Plate 3, Fig. 8-B) or of the uterus (Plate 3, Fig. 9) may be extensive. In the latter case there is in some places an abundance of smooth muscle fibers in the capsule itself. The thickening of the capsule of the kidney is sometimes noticeable even to the naked eye because of the whitish color of the surface. There is also a rich development of fibrous tissue in the mammary gland, sometimes, though very rarely, with formation of adenofibroma.

It is well known that the stroma of the prostate increases greatly under the influence of a prolonged treatment with estrogens, as reported by Parkes and Zuckerman (29), and De Jongh and associates (3). Loeb, Suntzeff, and Burns (24) have described the influence of estrogens on the stroma of other organs.

Discussion

From the observations reported in this paper, it is evident abdominal tumors, experimentally induced by estrogens, present all transitions from fibromyoma to fibroid, sometimes with an abundance of cells. As to their origin it is very likely that proliferation starts at different points of the serosa and in the proximity of lymphatic or vascular spaces. It is as yet not possible to say whether the celomic, lymphatic, or vascular endothelium becomes active or whether only cells underlying the endothelium enter into proliferation. The latter is more probable than the former. There can be no doubt that cells of the mesenchyme become activated so as to proliferate abundantly, under the influence of a prolonged treatment with estrogens. There is an accumulation of cells around the blood vessels even outside the fibroid, as in adipose tissue. The eventual results of these proliferative processes are, though probably not always, spindle-shaped cells, smooth muscle fibers, and collagenous fibers.

It is natural to question whether these experimental abdominal fibroids can be compared with the uterine fibroids of the woman. There are certain structural differences which we have already insisted upon. These differences are not coincident with species differences as shown by the fact that the only spontaneous uterine fibromyoma in the guinea pig which Lipschütz (11) had the opportunity to observe was structurally very similar to the spontaneous fibroids of the woman and very different from the experimental fibroids of the guinea pig. The great abundance of cells in some of the experimental tumors and the great tendency to invade certain organs has also to be mentioned here.

But one should not attribute too much importance to these structural and biological differences between experimental and spontaneous fibroids. One must not forget that in these experiments the tumor is induced under quantitative and time conditions which are probably very different from those in the body when a fibroid originates spontaneously. Quantities of estradiol no less than 100 to 150 times greater than the hysterotrophic or physiological dose are necessary to induce experimental fibroids. With esterified estradiol the difference between the hysterotrophic and the tumorigenic dose is smaller than with the free hormone (16, 30, 20), but here again we must insist that esterification overthrows completely the chronological conditions of the action of ovarian estrogens. No knowledge exists as yet on these fundamental quantitative and chronological questions in spontaneous tumorigenesis and one cannot expect for the moment to induce experimental fibroids structurally identical with the spontaneous ones.

The question has been raised whether the fibromyoma, experimental or spontaneous, is a neoplasm at all (8, 9). If autonomous growth; *i.e.*, ability to grow even when the stimulus that has led to its appearance is withdrawn (9), is an essential of true neoplasm,

benign or malignant, then spontaneous fibromyoma is not a tumor; fibromyoma in the woman ceases growing and begins to regress when the ovary ceases its endocrine function. There is no tumoral cell, or tumoral autonomy of spontaneous fibromyoma (29). The experimental fibromyoma also begins to regress when treatment is suspended and important structural changes take place (14). These changes, hyalinization and ossification, are similar to those in the fibromyoma of the woman in the menopause.

We were also unable to obtain permanent grafts of our experimental fibroids though they may persist for some time in the host into which they have been transplanted.

The question whether the fibromyoma, spontaneous or experimental, is a true neoplasm can be settled only on the broader basis of experimental tumorigenesis. If the fibromyoma is not a neoplasm, then neither is the uterine adenoma, since the latter also can be produced by injection of follicular hormones even with relatively small quantities (23). These probably will regress partially when treatment is suspended. But on the other hand, atypical epithelial growth induced in the uterus by prolonged administration of estrogens can eventually persist when administration is suspended (14) and can even become transplantable as shown by Gardner, Allen, Smith, and Strong (4). There is evidently a transition from proliferating epithelial cells without autonomous growth, to true tumoral epithelial cells. This transition is the fundamental problem of malignant neoplasms and of experimental tumorigenesis in general.

One may also question whether the great capacity of the experimental fibroid to invade certain neighboring tissues, which we have described, indicates transition from benign growth to malignancy. One should indeed keep in mind that a similar behavior of connective tissue can be seen also in other conditions of fibroblastic proliferation which has nothing to do with tumorigenesis, as in hepatic disease. But have we not overestimated the gap between localized tumorigenesis and general tissue reactions? Our own results with experimental extra-uterine fibroids may serve as a first step in dealing with this question.

Summary

The microscopic structure of experimental fibroids, uterine and extra-uterine, induced by a prolonged administration of estrogens, is described.

The participation of the cellular elements of the abdominal wall and of those of lymphatic and vascular spaces in the proliferative process is discussed.

The differences between experimental and spontaneous fibroids are emphasized, especially the abundance of cells in the experimental tumor and their capacity to infiltrate and destroy certain differentiated tissues such as pancreas, liver, striated muscle, and smooth muscle.

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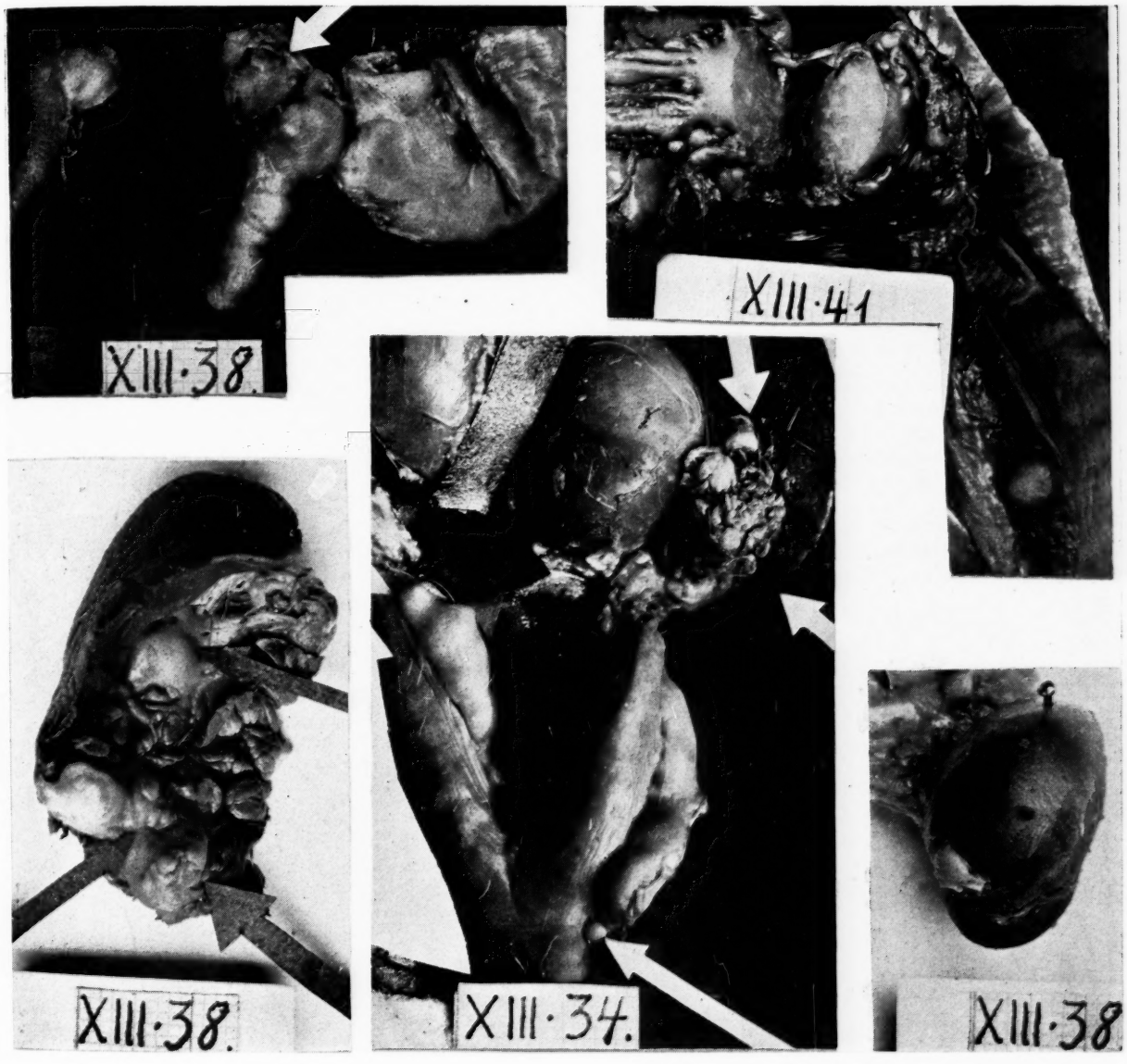


PLATE 1. FIBROID TUMORS AT VARIOUS SITES

FIG. XIII. 34. Large bilateral tumors of the mesometrium; fibrous masses in the apical region of the right uterine horn; apical tumor of the left uterine horn united with tumor of the hilum of the spleen, involving also the pancreas. The left kidney is displaced to the right by pressure of masses of tumor. Castrated female guinea pig which received 25 injections of 80 μgm. of estradiol monobenzoate in 63 days. Mag. × 1.5.

FIG. XIII. 38. Fibroid tumors from castrated female guinea pig treated in same manner as XIII. 34. Upper left, tumor of mesocolon and large tumor of the duodenum near the pylorus. Lower left, several large tumors at the hilum of the spleen. Lower right, right kidney with subserous fibroid near the lower pole and subserous tumor of the abdominal wall. Mag. × 1.5.

FIG. XIII. 41. Large fibroid tumor between spleen, pancreas, and stomach. The tumor has been cut across to show the diameter in the mid-region. Pedunculated uterine fibroid near the parametrium. Castrated female guinea pig treated as XIII. 34. Mag. × 1.5.

PLATE 2

FIGS. 1-A, 1-B, and 1-C. Sections from a large apical tumor of the left uterine horn, extending to the kidney and spleen and penetrating the pancreas. From castrated female guinea pig, II. 14, injected thrice weekly for 122 days with 80 μgm. estradiol benzoate (total 3.9 mgm.). Van Gieson stain. Mag. × 200.

FIG. 1-A. Loose and dense fibrous tissue in great disorder; cells scattered between the fibers.

FIG. 1-B. Bundles of smooth muscle fibers and fibrous tissue.

FIG. 1-C. Edematous fibrous tissue.

FIGS. 2-A and 2-B.—Sections from a tumor between the spleen and pancreas in castrated female guinea pig, II. 15, injected with doses of 80 μgm. estradiol benzoate (total amount 3.9 mgm.) during 122 days. Van Gieson stain.

FIG. 2-A. Portion of the tumor near the spleen, showing capsule consisting of flattened cells, fibroblasts (?) beneath the capsule and other cells separated by fibrous tissue. Mag. × 200.

FIG. 2-B. Portion of the tumor near the apex of the uterine horn, showing smooth muscle tissue in contact with adipose tissue and a Wolffian tubercle (?). Mag. × 45.

FIG. 3. Section from a fibroid between the spleen and stomach in female guinea pig, II. 38, injected during 81 days with doses of 80 μgm. estradiol benzoate (total amount 2.6 mgm.), showing abundance of cells of various types surrounded by fibrous tissue. Van Gieson stain. Mag. × 560.

FIGS. 4-A, 4-B, and 4-C. Sections from small nodules, "tumoral seed," on the serosal surface of the stomach in castrated female guinea pig, II. 17, injected with doses of 80 μgm. estradiol benzoate during 68 days (total amount 2.2 mgm.). Van Gieson stain.

FIG. 4-A. Abundant fibrous tissue between the tumor and muscle layer; penetration of fibrous tissue between muscle fibers. Mag. × 23.

FIG. 4-B. Two small nodules of "tumoral seed" on the serosal surface of the abdominal wall. Mag. × 10.

FIG. 4-C. Small cellular fibroid on the spleen, surrounded by adipose tissue. Mag. × 45.

FIG. 5. Section of small tumors on the spleen in a castrated male guinea pig, VIII. 15, injected with doses of 80 μgm. of estradiol benzoate-butyrate during 104 days (total amount 3.5 mgm.). Abundance of cells. Van Gieson stain. Mag. × 200.

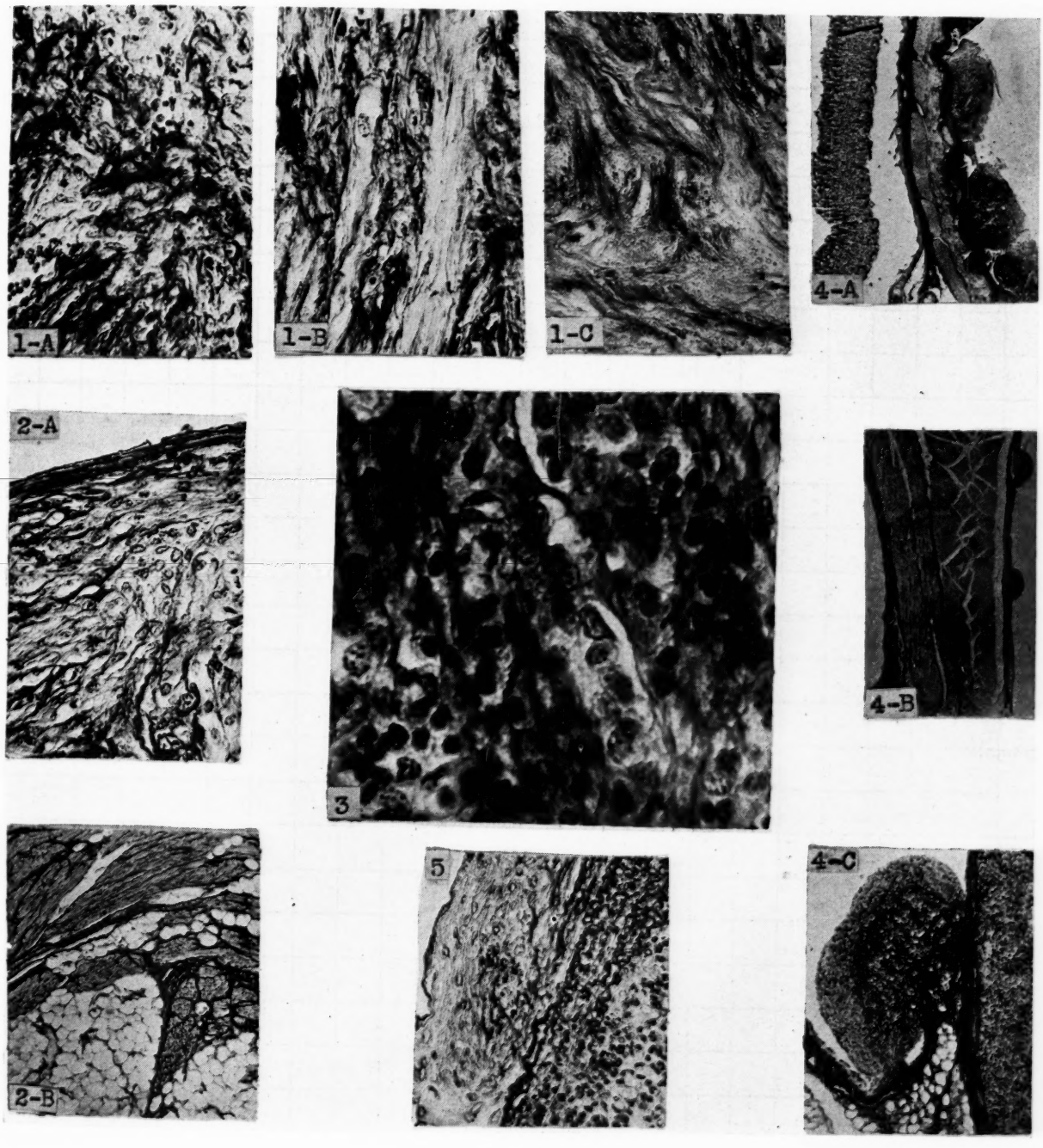


PLATE 2

PLATE 3

FIGS. 6-A and 6-B. Section of nodule of tumoral seed on the tunica albuginea of the testis in male guinea pig, VIII. 9, injected with doses of 80 μgm. of estradiol benzoate-butyrate during 99 days (total amount 3.3 mgm.). The nodule is poor in cells, does not contain muscle fibers and shows sclerotization. Van Gieson stain. Mag. × 200.

FIGS. 7-A and 7-B. Sections of spleen to show serosal surface. Van Gieson stain.

FIG. 7-A. Mesothelium on surface of the spleen in a normal female guinea pig, C.u. 13, weight 555 gm.

FIG. 7-B. Thickened mesothelial covering of spleen in male guinea pig, VIII. 15, treated as described in legend for Fig. 5. Mag. × 200.

FIGS. 8-A and 8-B. Sections through capsule of kidney. Van Gieson stain. Mag. × 200.

FIG. 8-A. Thin capsule in normal animal C.u. 13.

FIG. 8-B. Thickened capsule of kidney in male guinea pig, VIII. 6, injected with doses of 80 μgm. estradiol benzoate-butyrate during 87 days (total amount 2.8 mgm.).

FIG. 9. Section through thickened serosal surface of the uterus in castrated female guinea pig, V. 28, injected with doses of 400 μgm. of free estradiol during 123 days (total amount 21 mgm.). Thick layer of smooth muscle fibers beneath the mesothelium; dense fibrous tissue between this layer and the myometrium. Van Gieson stain. Mag. × 200.

FIGS 10-A, 10-B, 10-C, 10-D, 10-E, and 10-F. Sections of small tumors in the uterine wall of castrated female guinea pig, V. 28, injected with doses of 400 μgm. of free estradiol during 123 days (total amount 21 mgm.); same animal as in Fig. 9. Van Gieson stain.

FIG. 10-A. Muscular septum between uterine horns containing glandular cysts, showing blood-containing clefts in the muscle tissue, with 2 fibroids. Mag. × 10.

FIG. 10-B. Small fibroid lying in a cleft in the muscle tissue. The cleft is lined with endothelium. Dense fibrous tissue at the base of the fibroid. Mag. × 45.

FIG. 10-C. Small fibroid in the myometrium. The cleft is lined by endothelium. Spindle-shaped cells at the periphery of the fibroid, showing a sharp contrast between the tumor cells above the cleft and the muscle cells below the cleft. Mag. × 200.

FIG. 10-D. Cleft in the myometrium with small tumors separated from the muscle tissue by fibrous tissue. Mag. × 45.

FIG. 10-E. The same nodule showing difference between the apparently necrotic cells (above) and the cells of the myometrium (below). Mag. × 200.

FIG. 10-F. Same cleft as in Figs. 10-D and 10-E, showing necrosis and accumulation of pigment in the tumor nodule. Mag. × 45.

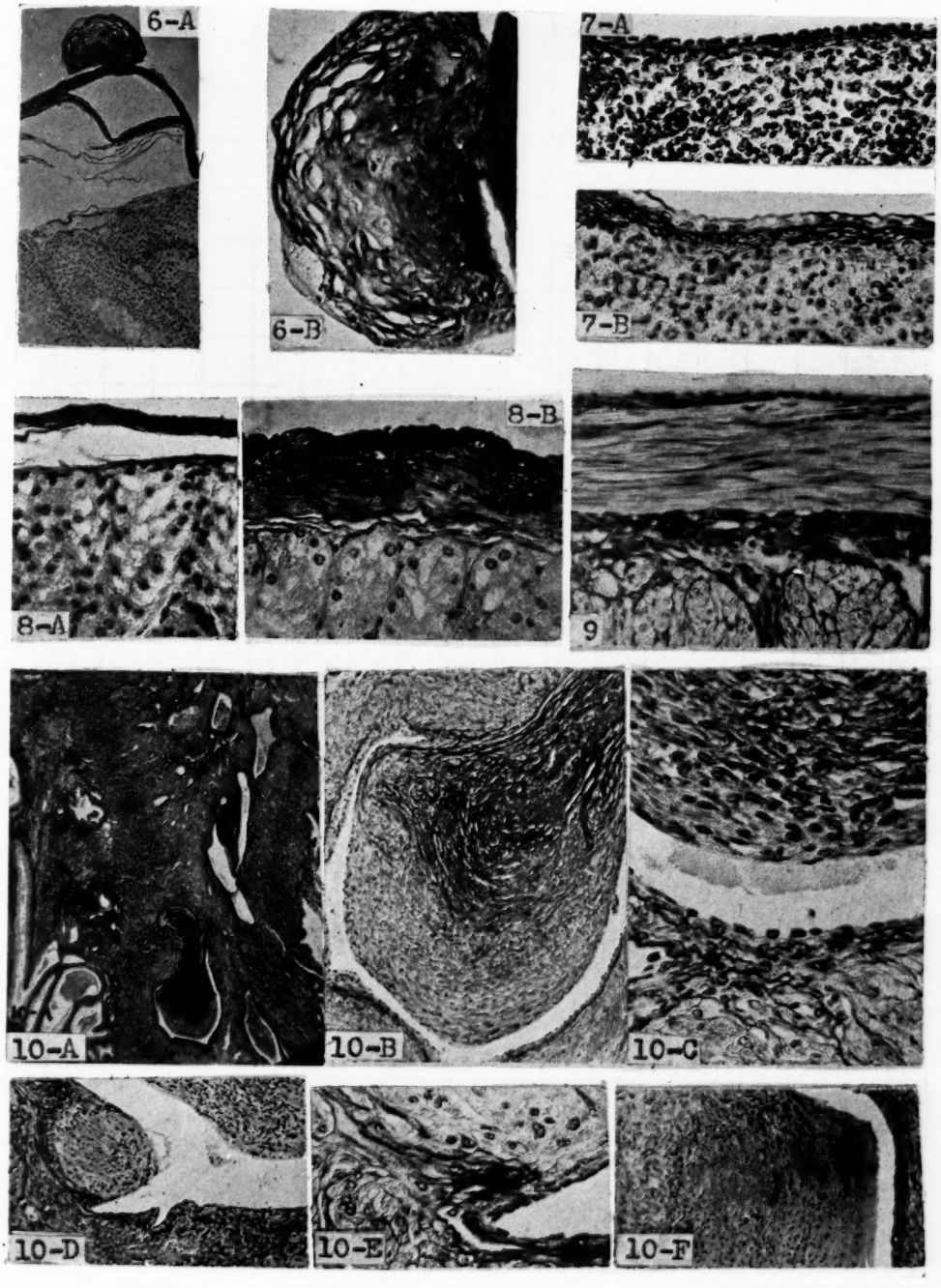


PLATE 3

PLATE 4

FIGS. 11-A, 11-B, and 11-C. Sections of fibroid between the spleen and pancreas in castrated female guinea pig, II. St. 2, injected with doses of 100 μgm. of stibestrol during 105 days (total amount 4.3 gm.). Van Gieson stain.

FIG. 11-A. Cells proliferating in the cleft between the tumor and the pancreas. Mag. × 100.

FIG. 11-B. Cells rich in cytoplasm on the surface of the tumor, with fibroblasts below the surface. Mag. × 200.

FIG. 11-C. Fibroblasts. Mag. × 450.

FIG. 12-A and 12-B. Section of apical tumor reaching the spleen in castrated female guinea pig, VI. 38, injected with doses of 80 μgm. dipropionate during 90 days (total amount 3.2 mgm.). Van Gieson stain.

FIG. 12-A. Cells rich in cytoplasm around a cleft (vascular space ?) in the center of the fibroid. Mag. × 300.

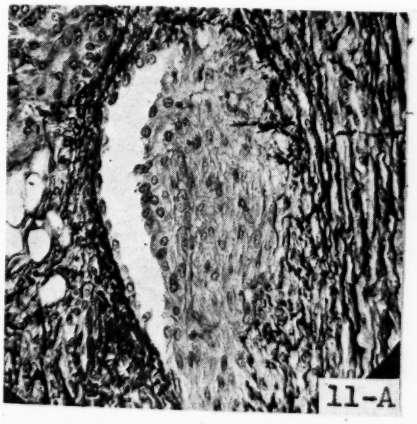
FIG. 12-B. Accumulation of cells in contact with adipose tissue and numerous blood vessels. Different types of cells may be due to proliferation of mesenchymal cells around the capillaries. Mag. × 560.

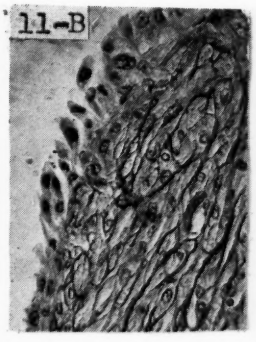
FIG. 13. Accumulation of cells rich in cytoplasm in adipose tissue near the hilum of the spleen in same animal as in Fig. 5. Mag. × 200.

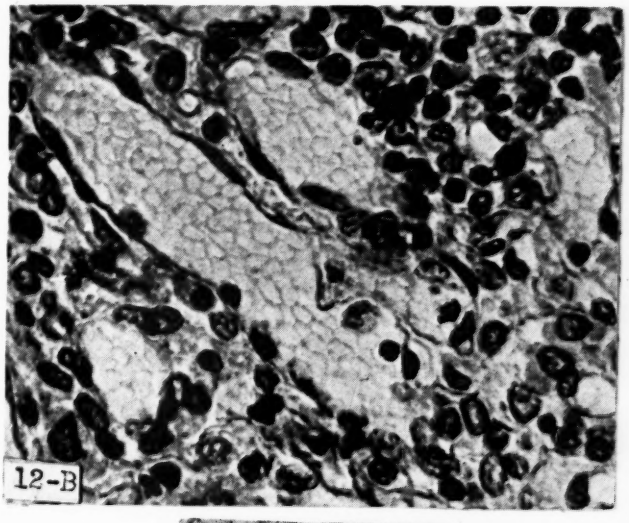
FIG. 14-A and 14-B. Sections through cleft between a tumor of the mesentery (right) and the intestinal wall (left) in castrated female guinea pig, I. 3, injected with doses of 40 μgm. estradiol benzoate during 121 days (total amount 2 mgm.). At other places the tumor adhered to the intestine and infiltrated the muscular coat. Van Gieson stain. Mag. × 240.

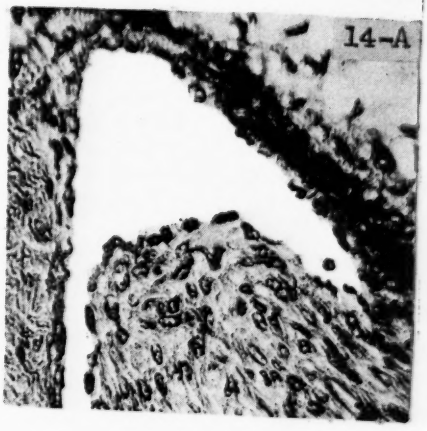
FIG. 14-A. Tip of nodule composed of spindle-shaped cells.

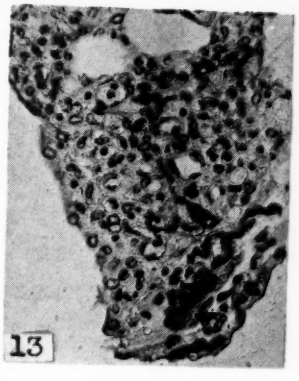
FIG. 14-B. The nodule in contact with the intestinal wall.











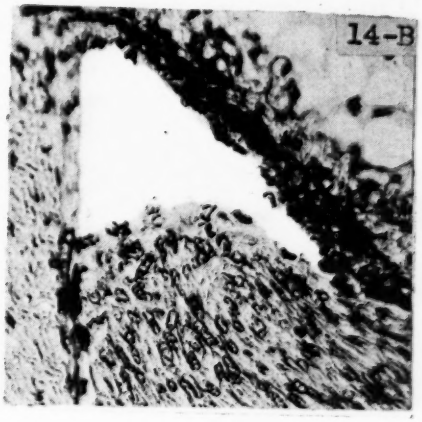


PLATE 4

PLATE 5

FIG. 15. Section of apical tumor infiltrating the pancreas, from same animal as in Fig. 1-A, showing clusters of pancreatic cells surrounded by fibrous strands of the tumor, causing disintegration and destruction of glandular tissue. Van Gieson. Mag. × 125.

FIG. 16. Section of subserous fibroid of the liver in castrated female guinea pig, II. 21, injected with doses of 80 μgm. estradiol benzoate during 80 days (total amount 26 mgm.). No sharp demarcation between the tumor and the liver. Van Gieson stain. Mag. × 560.

FIG. 17. Section of subserous tumor of uterus in castrated female guinea pig, V. 28, same animal as in Figs. 9 and 10, showing intermingling of muscle fibers of tumor and hypertrophied external layer of the myometrium. Van Gieson stain. Mag. × 45.

FIG. 18. Section showing infiltration of the muscular coat of the rectum by fibrous tissue of a pelvic tumor in castrated female guinea pig, I. 19, injected with 40 μgm. of estradiol benzoate during 125 days (total amount 2.1 mgm.). Hematoxylin and eosin stain. Mag. × 45.

FIGS. 19-A and 19-B. Sections of striated muscle infiltrated by fibrous tissue of a large fibromyoma in contact with the abdominal wall, in castrated female guinea pig, II. 14, same animal as in Fig. 1. Mag. × 100.

FIG. 19-A. Muscle fibers in transverse section surrounded by abundant fibrous tissue. Van Gieson stain.

FIG. 19-B. Muscle fibers in longitudinal section separated by fibrous tissue. Hematoxylin and eosin stain.

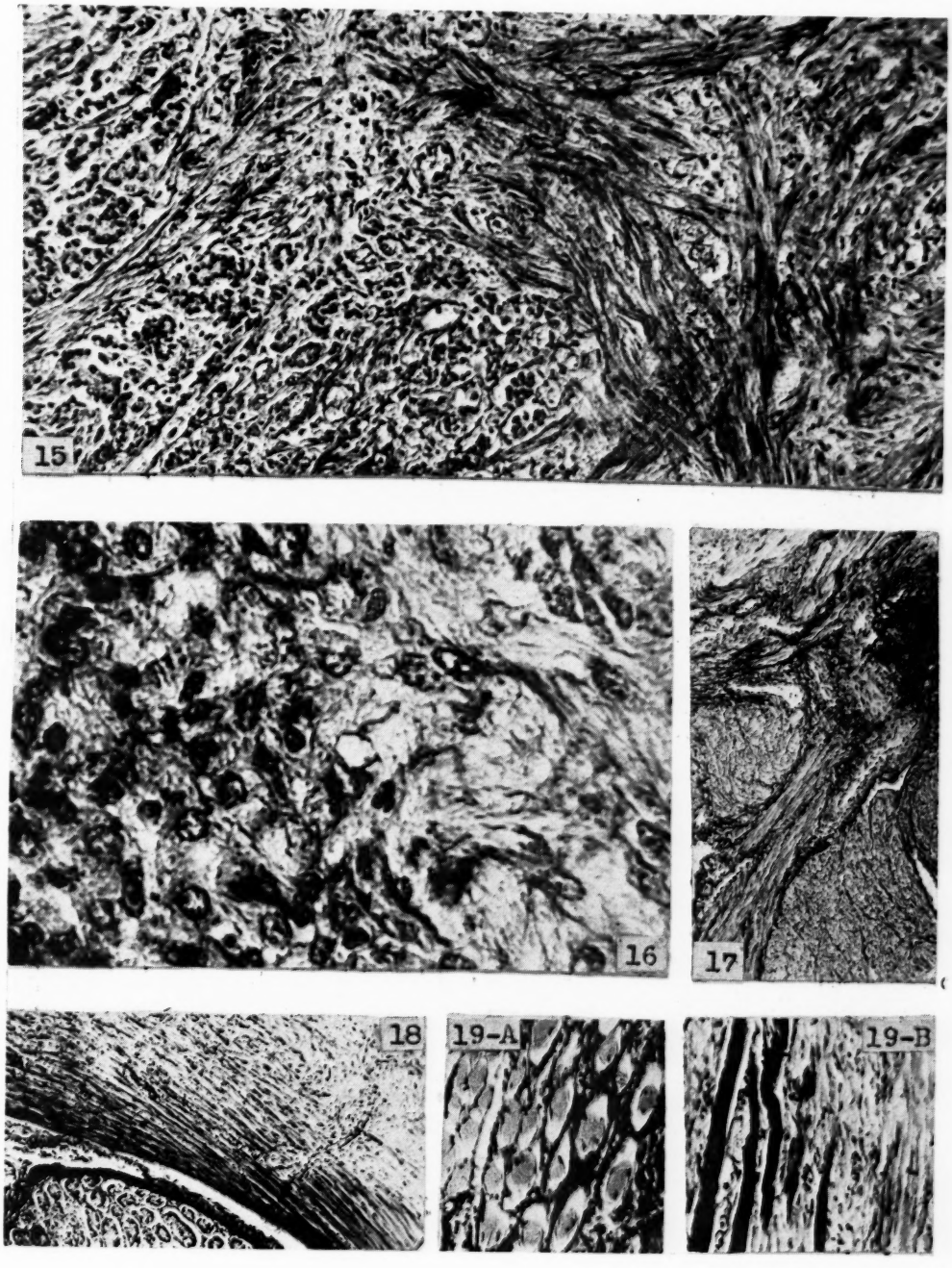


PLATE 5

Abstracts

Abstracts are grouped under the following main headings: Reports of Experimental Research, Clinical and Pathological Reports, Statistics, and Cancer Control and Public Health. Subheadings are used in accordance with the subjects of papers abstracted.

The initials of the abstractor are placed at the end of each abstract. Contributors of abstracts published in this issue are: S. Bayne-Jones, J. J. Bittner, A. Claude, Marie Duran-Reynals, W. U. Gardner, A. Kirschbaum, E. A. Lawrence, C. A. Pfeiffer, L. L. Waters, and G. Woolley.

REPORTS OF EXPERIMENTAL RESEARCH

Carcinogenic Compounds

ANDERVONT, H. B., and M. B. SHIMKIN. (Nat. Cancer Inst., Bethesda, Md.) Biologic testing of carcinogens. II. Pulmonary-tumor-induction technique. J. Nat. Cancer Inst., 1:225-239. 1940.

Thirteen carcinogenic compounds were injected intravenously into strain A mice. The incidence and latent periods of the induced pulmonary tumors were regular enough to make this method valuable as a quantitative biologic test for carcinogenic substances. A comparison of the carcinogenicity, however, of the same compounds for the lungs, skin, and subcutaneous tissues of mice showed that there was no quantitative or qualitative correlation in this respect. The carcinogens tested were 20-methylcholanthrene, 1,2,5,6-dibenzanthracene, 9 other related hydrocarbons, 2-amino-5-azotoluene, and colloidal thorium dioxide.—L. L. W.

EMMART, E. W. (Nat. Cancer Inst., Bethesda, Md.) The action of 2-amino-5-azotoluene in the production of liver tumors of rats and the behavior of these tumors in vitro. J. Nat. Cancer Inst., 1:255-272. 1940.

Rats of the Wistar, Osborne-Mendel, and Buffalo strains were fed purified 2-amino-5-azotoluene for periods up to 614 days. The diet included, besides the dye, 15.0% whole milk powder, 75.47% ground wheat, 4.0% dried yeast, 4.0% cod-liver oil and salt mixture. The increase in weight of the rats on the experimental diet was much less than those of the controls. For the first 7 months of dye feeding, changes in the liver consisted of injury, regeneration, and hyperplasia. The first gross tumor was observed at 211 days. Between 301 and 614 days, 81 of 91 animals showed gross tumors and all of 33 rats examined between 451 and 614 days had gross liver involvement.

Microscopically, the tumors were mostly hepatomas up to 500 days, but thereafter 4 of 7 rats had anaplastic liver cell tumors classed as carcinoma. Tissue injected from 9 hepatomas and 2 carcinomas into other rats failed to grow.

In vitro, liver tissue from normal embryonic, young and adult rats were studied as a base line for comparisons with hepatomatous tissues. Early embryo liver was kept alive 133 days, but the survival time decreased with the age of the rat. With normal adult rat liver, there was very little epithelial proliferation. Tissue cultures of hepatomas showed a great increase in growth over the normal liver controls. This growth increase continued for a limited time only and no culture of hepatoma survived more than 61 days. Tissue taken from areas adjacent to tumors showed a slight increase in growth over the controls although this growth was probably limited to fibroblasts and endothelium. One culture of carcinoma showed excellent growth after 94 days and was lost only because of infection. The cytology of this tumor *in vitro* and *in vivo* is described.—L. L. W.

LORENZ, E., and H. L. STEWART. (Nat. Cancer Inst., Bethesda, Md.) Squamous cell carcinoma and other lesions of the fore-stomach in mice, following oral administration of 20-methylcholanthrene and 1,2,5,6-dibenzanthracene. J. Nat. Cancer Inst., 1:273-276. 1940.

In a series of mice of strains C57 brown, C57 black, C3H, A and A backcross ingesting methylcholanthrene or dibenzanthracene emulsions over a period of 9 months, 10 developed squamous carcinoma of the forestomach and 30 developed hyperplastic changes in the gastric mucosa. All the gastric carcinomas arose in strain A and strain A backcross mice receiving methylcholanthrene emulsions. A mineral oil emulsion seemed more effective than one prepared with olive oil. Thirteen of the animals with gastric lesions showed adenocarcinoma of the small intestine as well. Metastases from the gastric tumors were found in 3 cases in the mesentery, pancreas, mesenteric lymph nodes, spleen, diaphragm, lungs, and chest and abdominal walls.—L. L. W.

SHIMKIN, M. B. (Nat. Cancer Inst., Bethesda, Md.) Biologic testing of carcinogens. I. Subcutaneous injection technique. J. Nat. Cancer Inst., 1:211-223. 1940.

A list of the factors to be considered in such biologic testing of carcinogens and related substances includes the following: I. Experimental animal: A. Species, B. Strain, C. Age, D. Diet, E. Condition of animals, 1. Intercurrent infection, 2. Ulceration at injection site, F. Environmental conditions. II. Carcinogen: A. Chemical structure, B. Purity, C. Physical state, D. Vehicle (solvent), E. Dose and concentration. III. Mode of administration: A. Route, B. Number of injections, C. Site. IV. Interpretation of results: A. Diagnosis, 1. Gross diagnosis, 2. Histologic examination, 3. Transplantation, B. Presentation, 1. Number of animals, 2. Average, 50%-latent time or carcinogenic index.

The author's summary follows: "The carcinogenicity of any given compound is modified by so many factors that it is necessarily a relative term, applicable only to the specific conditions of the reported experiment.

"As one standard test, the subcutaneous-injection technique into mice is recommended. Several widely divergent doses of the chemical, from 0.01 to 10 mgm., are dissolved in a solvent of known chemical composition, such as tricaprylin. A single subcutaneous injection into the right axilla is made into C3H male mice, at least 20 per group. The animals are 2 to 3 months of age and are maintained under constant conditions of diet and environment. The presence of tumor is determined by weekly palpatory examination. As soon as a growing hard mass is detected and has reached appreciable size, it is removed for histologic examination. Detailed data for the incidence and the latent periods of the neoplasms are presented."—L. L. W.

SHIMKIN, M. B., and J. LEITER. (Nat. Cancer Inst., Bethesda, Md.) Induced pulmonary tumors in mice. III. The role of chronic irritation in the production of pulmonary tumors in strain A mice. J. Nat. Cancer Inst., 1:241-254. 1940.

In an effort to determine if possible the role played by chronic irritation in the production of lung tumors, strain A mice were

given a single intravenous injection (5 mgm.) of finely ground arsenopyrite, chromite, thorite, or quartz (1 mgm.). These ores produced chronic inflammatory lesions in the lungs but no tumors within 6 months. Furthermore, they seemed to have no effect upon the number of tumors occurring after the intravenous injection of 0.1 mgm. of 20-methylcholanthrene. The tumors produced by this carcinogen did not arise more frequently in the foci of chronic inflammation than elsewhere in the lung.

A benzene extract of soot from a chimney burning soft coal produced spindle cell sarcomas and pulmonary tumors in C3H male mice. The intravenous injection of 2.5 mgm. of the unextracted soot increased the incidence of primary pulmonary tumors in strain A mice. The authors point out the possible industrial hazards indicated by such studies—L. L. W.

SPENCER, R. R., and M. B. MELROY. (Nat. Cancer Inst., Bethesda, Md.) Effect of carcinogens on small free-living organisms. I. Eberthella typhi. J. Nat. Cancer Inst., 1:129-134. 1940.

Pure cultures of *E. typhi* were carried through 240 daily transfers into 10 cc. of broth containing 12 mgm. of methylcholanthrene. Other cultures of *E. typhi* were transferred 192 consecutive days to tubes of broth into which a 10 mgm. radium-emanation needle had been inserted. Much care was given to control material. After these long periods of exposure to methylcholanthrene and radium emanations, "no structural, functional, or immunological changes were observed other than an immediate stimulation of the rate of cell division which had been previously reported by other investigators." These negative results contrast strongly with the effect of methylcholanthrene and radium on a strain of *Paramecium*. A report of this latter experiment will be published later.—L. L. W.

STEINER, P. E. (Univ. of Chicago, Chicago, Ill.) A cancerogenic tissue extract from human sources. Science, 92: 431-432. 1940.

An extract of the saponifiable residue prepared from the liver of patients with carcinoma was dissolved with sesame oil and injected in mice. Thirteen tumors, all spindle or polymorphous cell sarcomas, were observed in a total of 56 mice. Similar tumors had never been observed in 2,000 controls. The nonsaponifiable residue of the liver from 7 persons who had died from nonneoplastic diseases and the nonsaponifiable residue from carcinoma tissue failed to produce tumors.—J. J. B.

Hormones

BECK, F. F., and J. C. KRANTZ, JR. (Dept. of Pharmacol., Sch. of Med., Univ. of Maryland, Baltimore, Md.) Tumor glycolysis. IV. The effect of feeding thyroid supplemented by thiamin chloride on the growth and glycolysis of Walker sarcoma 319 in rats. Cancer Research, 1:188-190. 1941.

An experiment was made to determine whether or not the correction of a deficiency of vitamin B_1 in hyperthyroidism would alter the effect of the hormone on tumor growth. No consistent difference was observed in the growth of Walker sarcoma 319 in the 5 experimental groups; namely, (a) thyroid-fed, (b) thyroid-fed plus vitamin B_1, (c) controls, (d) thyroidectomized, (e) weight control. No consistent differences in the pH drop or lactic acid production of the tumors was noted among the groups. The state of hyperthyroidism in which the deficiency of vitamin B_1 was corrected had no significant effect on the growth and metabolism of Walker sarcoma 319.—Authors' summary.

BRADBURY, J. T. (Univ. of Michigan Med. Sch., Ann Arbor, Mich.) Permanent after-effects following masculinization of the infantile female rat. Endocrinology, 28:101-106. 1941.

Seven female rats receiving 2 R.U. of antuitrin-S daily from the 6th to the 24th day of life and 17 receiving 0.25 mgm. testosterone propionate from the 6th to the 38th day were characterized by a continuous estrous smear and only follicular development of the ovary. When the same androgen treatment was started on the 4th day the results were the same except that development of the lower vagina was inhibited in 13 of 16 rats treated. Most of the experimental pathology produced by prolonged injection of estrogens was obtained on these constant estrous rats. Marked vaginal and cervical hypertrophy were observed in 2 animals and in 2 cases gross pyometra occurred. Tubo-ovarian abcesses were numerous. The infectious abcesses were associated with the prolonged estrous condition and were the result of continuous endogenous estrogen production. The after effects of APL or androgen treatment were probably mediated by altering the hypophyses to the male type.—C. A. P.

BURACK, E., J. M. WOLFE, W. LANSING, and A. W. WRIGHT. (Depts. of Anat. and Path., Albany Med. Coll., Union Univ., Albany, N. Y.) The effect of age upon the connective tissue of the uterus, cervix, and vagina of the rat. Cancer Research, 1:227-235. 1941.

The uteri, cervices, and vaginae of rats of the Albany (A-S) and Vanderbilt (V-S) strains, killed at ages ranging from 30 to over 800 days, were examined histologically to determine the effects of age and endocrine factors upon connective tissue. As age advanced the collagenous tissue increased in amount and density in the uterus, cervix, and vagina of the normal rat. In the endometrium this increase was greatest from the immature to the 3-month stage. Thereafter the deposition of collagen varied, influenced not only by age but also by the sexual activity of the animal. The observations tended to associate the deposition of collagenous tissue in the accessory reproductive organs of the rat with the long-continued unmodified influence of estrogen in the nonpregnant state. Beginning with 1 year of age, connective tissue increased in the muscle layers of the uterus. Large amounts of collagenous tissue developed around the uterine arteries, especially between the muscle coats. The paper is illustrated with 8 photomicrographs and 1 diagram.—S. B-J.

GARDNER, W. U. (Dept. of Anat., Yale Univ. Sch. of Med., New Haven, Conn.) The breaking strength of femurs of mice receiving estrogens. Proc. Soc. Exper. Biol. & Med., 45:230-232. 1940.

The femurs and other bones, especially of the appendicular skeleton, of mice which received estrogens had greatly thickened walls. Following prolonged treatment the marrow was largely or completely replaced by new osseous tissue (Proc. Soc. Exper. Biol. & Med., **37**:678. 1938; **38**:599. 1938; Anat. Rec., **76**: suppl. 22. 1940). The breaking strength of femurs of 30 estrogen-treated mice averaged 2,499 gm. This was 844 gm. greater than that of untreated controls. Details of diet, estrogen treatment, and ages of mice are given.—S. B-J.

GARDNER, W. U. (Dept. of Anat., Yale Univ. Sch. of Med., New Haven, Conn.) Growth of the mammary glands in hypophysectomized mice. Proc. Soc. Exper. Biol. & Med., 45: 835-837. 1940.

Slight growth of mammary glands of some hypophysectomized male mice was induced within 15 days by the injection of desoxycorticosterone acetate, progesterone, and estradiol propionate. A more extensive and more rapid proliferation of the mammary ducts of hypophysectomized mice occurred when desoxycorticosterone acetate or progesterone was injected with the estradiol dipropionate.—Author's summary.

GARDNER, W. U. (Dept. of Anat., Yale Univ. Sch. of Med., New Haven, Conn.) Inhibition of mammary growth by large amounts of estrogens. Endocrinology, 28:53-61. 1941.

The inhibitory effects of large doses of estrogen on mammary gland development were tested on 67 male C3H mice, 1 young male and 6 immature or young mature female monkeys (*Macacus rhesus*), and 4 female dogs (fox terriers). In the mice some

inhibition followed prolonged administration of weekly doses of 50γ of estradiol benzoate while almost complete inhibition resulted from the same treatment with 50γ of estradiol dipropionate. Of the 17 mice receiving 50γ of estradiol dipropionate weekly, only 1 developed a mammary carcinoma while, associated with better mammary growth, tumors developed in more than 80% of the cases (normal incidence for multiparous female C3H mice) when 25γ of estradiol benzoate weekly was administered. Inhibition of the mammary glands followed prolonged treatment with weekly doses of over 400γ of estradiol benzoate to monkeys and of 1333γ to 5333γ to the 4 dogs. Much higher doses administered for brief periods to 3 of the dogs failed to produce any mammary growth.—C. A. P.

HAAGENSEN, C. D., H. T. RANDALL, and R. AUCHINCLOSS. (Surgical Pathol. Lab., Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.) Failure of thyrotropic pituitary hormone to prevent spontaneous mammary cancer in mice. Proc. Soc. Exper. Biol. & Med., 45:820-823. 1940.

An experiment of Cramer and Horning (Lancet, **2**:72. 1938) was repeated on a larger scale, using the same strain of mice, RIII, and the same pituitary extract, ambinon, purchased from N. V. Organon Co., Oss, Holland. This impure preparation contained 50 units of gonadotropic hormone in addition to 150 units of thyrotropic hormone per cc. Thirty-three mice were injected and 100 mice were used as controls. The treatment was begun when the mice were just under 2 months of age and 0.1 cc. of the hormone solution was injected twice weekly for 15 months. Twenty-nine mice survived at least 6 months and 13 were alive 15 months after the start of the experiment. The results with the 29 mice are presented in a table. This long-continued subcutaneous injection of an anterior pituitary preparation containing thyrotropic hormone and some gonadotropic hormone did not definitely affect the incidence of mammary carcinoma in female mice of the RIII strain. S. B-J.

STEINKAMM, E. (Universitätsfrauenklinik der Chariti in Berlin.) Tierexperimentelle Untersuchungen über die Folgen longdauernder Zufuhr grosser Mengen Dioxydiäthylstilbene. Experimental investigation on the results of long continued administration of large amounts of stilbestrol. Archiv. f. Gynäk., 170:307-316. 1940.

Stilbestrol in oily solution in amounts of 0.5 to 3.0 mgm. was injected subcutaneously into 40 castrate female rats. About 1/3 of the rats died within 18 days. Eight rats lived over 13 1/3 weeks. All showed marked estrous reactions. Some uterine metaplasia and pyometra appeared. Body weight and blood cell counts decreased. Liver damage was not observed. The adrenal glands showed marked hyperemia. The rats were compared with estradiol-treated animals.—W. U. G.

WOOLLEY, G., E. FEKETE, and C. C. LITTLE. (Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine.) Differences between high and low breast tumor strains of mice when ovariectomized at birth. Proc. Soc. Exper. Biol. & Med., 45: 796-798. 1940.

The following differences were noted between 2 high and 1 low breast tumor strains of mice when ovariectomized at birth and examined in later life (beyond 1 year of age). The high tumor JAX dba and JAX C3H strains showed stimulated uterus, vagina, and mammary glands. Mammary adenocarcinomas appeared. The adrenal glands exhibited extensive nodular, hyperplastic areas. All of the above organs of the low tumor JAX C57 black strain remained essentially unstimulated. No mammary gland tumors appeared.—G. W.

Viruses

SHEMIN, D., E. E. SPROUL, and J. W. JOBLING. (Dept. of Path., Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.) Studies of the transmissible agent of the Rous sarcoma I. Precipitation with basic proteins. J. Exper. Med., 72:697-705. 1940.

The paper presents an attempt to purify the filtrable agent transmitting chicken tumor I by the medium of a precipitate which formed when a basic protein was added to the tumor extract. A first step in the procedure was to reduce the viscosity of crude extracts by treatment at 37° C. with a pneumoccocus enzyme (hyaluronidase), which hydrolyzes a tumor polysaccharide of high viscosity. Subsequently, the extract was passed through a Berkefeld V filter. A proper amount of neutral papain solution was added to the filtrate, the precipitate washed with cold water and finally redissolved in 3% NaCl solution. The crude papain complex was reprecipitated twice by tenfold dilution with distilled water and redissolved in 3% NaCl. Fifty to 70% of this purified papain complex was papain, along with a certain amount of lipids and possibly nucleic acid. The smallest amount of material which was required to produce a tumor, when injected into 3 week old chicks was 10^{-9} gm. of the purified papain complex, in terms of nitrogen, as compared with 10^{-7} gm. of the original tumor filtrate. The purified papain complex was examined in the Tiselius apparatus and fractions obtained after 7 to 11 hours electrophoresis were tested for tumor producing activity. The material from the cathode compartment was inactive and consisted mainly of papain. The tumor producing power of the material from the anode compartment was not higher than that of the material from the "middle" compartment, and was equal to that of the untreated preparation, the minimal active dose in each case corresponding to about 10^{-7} gm. nitrogen. Study of the electrophoretic pattern indicated that the purified fraction contained other material beside the basic protein and the tumor agent.—A .C.

Genetics

ANDERVONT, H. B. (Nat. Cancer Inst., Bethesda, Md.) Further studies on the susceptibility of hybrid mice to induced and spontaneous tumors. J. Nat. Cancer Inst., 1:135-145. 1940.

F1 hybrid mice derived from C3H mothers and I and Y strain fathers together with backcross generations procured by mating the hybrids to their parental strains were tested for their susceptibility to induced dibenzanthracene tumors, to induced pulmonary tumors, and to the occurrence of spontaneous mammary cancer. It was found that susceptibility to all 3 types of tumor is inherited in a dominant manner and that multiple, modifying genetic factors are involved. Although the susceptibility of the 3 inbred strains to induced and spontaneous tumors was known, it was impossible to predict the degree of susceptibility of their hybrid offspring because of these modifying factors.—L. L. W.

ANDERVONT, H. B. (Nat. Cancer Inst., Bethesda, Md.) The influence of foster nursing upon the incidence of spontaneous mammary cancer in resistant and susceptible mice. J. Nat. Cancer Inst., 1:147-153. 1940.

The experiments reported in this paper were designed to ascertain whether the ingestion of milk from low tumor strain mothers would lower the incidence of breast tumors in strain C3H females and whether the ingestion of milk from a high tumor strain, C3H, would produce breast tumors in females of strains in which the spontaneous occurrence of such tumors is low. The resistant strains used were I, Y, C, and C57 black. Foster nursing of C3H mice by C, Y, and I females reduced the incidence of breast tumors in the C3H mice from 100% at an age of 8.4 months to approximately 50% at an average age of 12 months. The reverse experiment, in which I, Y, and C57 black mice were fostered by C3H females increased the tumor incidence in all 3 strains from 0 to nearly 15%. C mice fostered by C3H females showed an increase of tumor incidence from

0 to 64%; C57 black × I hybrids nursed by C3H females from 0 to 71%. The experiments were controlled by using non-fostered litter-mates.—L. L. W.

BITTNER, J. J. (Nat. Cancer Inst., Bethesda, Md., and Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.) Breast cancer in mice as influenced by nursing. J. Nat. Cancer Inst., 1:155-168. 1940.

The author reviews his own extensive work and that of others on inherited tendencies toward breast cancer in mice. By correlating the results of successive experiments (which are given in some detail), he is able to ascribe the occurrence of breast cancer in mice to the combined activity of at least 3 etiological influences: (1) The milk influence. This extrachromosomal, maternal factor is transferred in the milk to the progeny in the first 24 hours of life. A similarly active substance has been found in normal tissues and organs transplanted from 1 strain to another. (2) Inherited susceptibility. Studies of breast tumor ratios in hybrid animals are in accord with the genetic theory that this susceptibility is inherited as a single dominant factor. (3) Ovarian hormonal stimulation of the mammary gland.—L. L. W.

BITTNER, J. J. (Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.) Further studies on active milk influence in breast cancer production in mice. Proc. Soc. Exper. Biol. & Med., 45:805-810. 1940.

The paper presents a condensed report of experimental investigation of the effect of foster nursing upon the incidence of spontaneous mammary cancer in several strains of mice and tests for the presence of the milk influence in various organs. A bibliography of publications of the author and others working on this problem since 1936 is included with a review.

The "breast cancer producing influence" is an actual active "influence" present in the milk of high cancer stock females. It is probably present and active during the entire lactation period. The active influence may be transferred by the inoculation of spleen, thymus, and lactating mammary gland tissue from cancerous stock animals. The active influence is probably not present (or has been destroyed) in the liver of high tumor stock mice. The active influence may be given to 4-week old females by feeding by mouth milk obtained from lactating females of a cancerous stock. Fostered females of low breast tumor strains need not develop breast tumors to transfer the active milk influence by nursing. An active milk influence may be necessary for the development of induced estrogenic breast tumors.—S. B-J. and Author's summary.

HAMMETT, F. S. (The Lankenau Hosp. Research Inst., Philadelphia, Penn.) Genetics, chemistry and cancer. J. Hered., 31:511-513. 1940.

Chemical mechanisms, chemical participants, and chemical determinants are used by heredity in the production of non-malignant structures. For the eventual understanding of the abnormal growth, cancer, it is necessary to learn more about normal growth and its chemistry.—J. J. B.

van GULIK, P. J., and R. KORTEWEG. (Netherlands Inst. of Cancer Research, Amsterdam.) The anatomy of the mammary gland in mice with regard to the degree of its disposition for cancer. Proc. Nederl. Akad. van Wetenschappen, 43:891-900. 1940.

The authors determined the incidence of mammary tumors in virgin females of the Murray-Little strain of dilute brown mice (to be called "D") and the C57 stock of Little (to be called "B"). Eighty-five per cent of the control mice of the D stock had cancer and 9% of the D females fostered by B mothers. The incidence in mice of the B stock (194 mice) was 0%, and when fostered by D females was 13%. Mice of the 0_{20} Leeuwenhoekhuis strain had an incidence of 1%. The incidences in F1 females were: D♀ × B♂, 69% in controls, 4% in fostered (by B♀♀); B♀ × D♂, 2% in controls, 46% in fostered (by D♀♀). Backcross generations were made by mating hybrids having D stock maternal parents to males of the B stock and hybrids having maternal parents from the B stock to males of the D strain. These matings were continued for 11 generations to the paternal stocks and in each group the incidence of mammary tumors was 1%.

From these results the workers concluded that the extrachromosomal factor was independent of and not a product of the genetic composition. The genetic susceptibility for mammary cancer is of little importance if the extrachromosomal factors are inactive. Because the incidence was lower in backcross mice of the 11th generation than in mice of the B stock fostered by D females, they believed that the extrachromosomal factors might become inactive after a number of generations.

Mice in the same stage of diestrus were selected for study of the architecture of the mammary glands. The structure of the glands of the mice of the B stock was described as resembling a tree in winter and the glands of the females of the D stock resembled a budding tree in spring,—buds along the primary ducts and at the beginning of the secondary ducts. Females of the D strain fostered by B females had primary ducts of the normal D type but the gland tree possessed characteristics of the B type. The B females fostered by females of the D stock had primary ducts of the normal B type. The gland tree, on the whole, had the architecture of the B type but some areas were identical with the D type. The architecture of the primary ducts was believed to be determined by the plasma, and/or uterus factor, and the structure of the gland tree determined by the genetic and the extrachromosomal factors,—milk, plasma, and uterus factors.

In different types of mice there is a correlation between the architecture of the mammary gland and the degree of the disposition for mammary cancer.—J. J. B.

WRIGHT, F. H. (Rockefeller Inst. for Med. Research, New York, N. Y.) Effect of foster-nursing upon inborn resistance of mice to St. Louis encephalitis. Proc. Soc. Exper. Biol. & Med., 45:871-873. 1940.

Foster nursing, by which the incidence of mammary tumors in mice may be reduced, exerted no influence upon the course of, or mortality rate of, encephalitis.—J. J. B.

Physical Factors

GOLDFEDER, A. (Cancer Division, Dept. of Hospitals, New York City, and New York Univ. Med. Coll., New York, N. Y.) The effects of reduced temperatures upon the growth and metabolic changes of sarcoma 180 grown in vivo. Cancer Research, 1:220-226. 1941.

Mice of the CFX strain inoculated with Crocker mouse sarcoma 180 were refrigerated at 5 to 7° C. intermittently or continuously from 8 to 48 hours. Tumors disappeared completely in 5 mice; in others a small decrease or temporary arrest of tumor growth occurred. Tumors which had attained relatively large size 16 to 18 days after inoculation were not affected by the reduction of temperature of the tumor-bearing animals. Metabolic studies using tissue slices failed to show significant changes in the oxygen consumption, respiratory quotients, and aerobic glycolysis of sarcoma 180 excised from refrigerated mice as compared with the tumor excised from control mice. The author concluded that "the high mortality rate, the comparatively few successful results, and the unaffected viability of tumor cells, indicate the inadequacy of refrigeration in the treatment of malignant growths."—S. B-J.

Radiation

PECHER, C. (Crocker Radiation Lab., Univ. of California, Berkeley, Calif.) Biological investigations with radioactive

calcium and strontium. Proc. Soc. Exper. Biol. & Med., 46: 86-91. 1941.

Quantitative data are presented showing the uptake of radioactive calcium and strontium in bone, muscle, skin, hair, digestive tract, liver, and other viscera after intravenous injections of lactate solutions into adult white mice. The excretion of radioactive calcium and strontium was studied. Comparative data are given on the uptake of strontium chloride, lactate, and gluconate. The selective fixation of radioactive strontium in the skeleton, the suitable energy of its beta-rays (1.5 million electron-volt), its half life of 55 days, and the innocuousness of small doses provide a specific method of irradiation of the skeleton. The experiments furnished 2 practical findings: (1) a method of selective irradiation of the skeleton for therapeutic purposes, and (2) the secondary production of appreciable amounts of a long-lived radioactive yttrium, the most likely among the artificially made radioactive elements now known, which may be substituted for radium as a penetrating gamma-ray source. The paper is illustrated with autographic pictures of bones containing radioactive phosphorus and strontium.—S. B-J.

PECHER, C., and J. PECHER. (Crocker Radiation Lab., Univ. of California, Berkeley, Calif.) Radio-calcium and radio-strontium metabolism in pregnant mice. Proc. Soc. Exper. Biol. & Med., 46:91-94. 1941.

Radioactive strontium and calcium have been used to study the mineral metabolism in mice during pregnancy. It appears that part of the calcium and strontium previously fixed in the skeleton of mice migrates to the fetus during the last days of pregnancy and to the offspring through the milk. When radioactive strontium lactate is injected intravenously into lactating mice and cows, appreciable amounts are excreted in the milk.—Authors' summary.

Biochemistry and Nutrition—Chemotherapy

BISCHOFF, F., and M. L. LONG. (Chem. Lab., Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.) The influence of terminal B avitaminosis with attending low body temperature upon the growth characteristics of sarcoma 180. Cancer Research, 1:217-219. 1941.

By producing B avitaminosis in mice of the Marsh-Buffalo strain, the inguinal skin temperature was reduced 2.5° to 16° C. below normal for periods ranging from 60 to 98 hours. The growth of subcutaneous transplants of sarcoma 180, which was completely arrested or markedly retarded during the period of avitaminosis with attending lowered body temperature, was resumed at a normal rate on return of the mice to a normal nutritional state.—Authors' summary.

HAMMETT, F. S. (Research Inst., Lankenau Hosp., Philadelphia, Pa.) l-Proline and tumor incidence in mice. Proc. Soc. Exper. Biol. & Med., 45:601-602. 1940.

From the time of the first appearance of mammary tumor until death, 78 mice were given daily by intrascapular subcutaneous injection 0.2 cc. of M/125 solution of l-proline in distilled water. The controls were 76 untreated mice kept under similar conditions. The mice injected with l-proline had more multiple primary tumors than the controls. A second tumor appeared in 56.4% of the proline-treated group, and in 44.7% of the controls. A third tumor appeared in 20.5% of the proline series and in 13.2% of the controls; and a fourth tumor developed in 9.0% in the proline-treated mice and in 2.6% of the controls. The author emphasizes that these experiments deal with *more* instead of *larger* tumors and indicate that the tumor incidence in mice is enhanced by l-proline probably through its favoring action in some process concerned in cellular differentiation.—S. B-J.

HAVEN, F. L. (Univ. of Rochester, Rochester, N. Y.) The rate of turnover of the lecithins and cephalins of carcinosarcoma 256 as measured by radioactive phosphorus. J. Nat. Cancer Inst., 1:205-209. 1940.

Chemical methods were used to determine the amounts of phospholipids present in rat carcinosarcoma 256. It was found that the ratio of lecithin to cephalin was 45:55, comparing favorably with a 40:60 ratio previously determined in the same laboratory. Also, after giving rats a tracer dose of radioactive phosphorus, the activity of the phospholipid fractions of the tumors was observed at intervals up to 20 days. The activity of the lecithins reached a peak after 30 hours and the cephalins after 40 hours. Thereafter, the tagged phosphorus began to disappear from both fractions at about the same rate. It was felt that the rapid rate of turnover of lecithin indicated that its function in the tumor was predominantly metabolic, while the cephalin was more concerned with structure.—L. L. W.

LAVIK, P. S., and C. A. BAUMANN. (Dept. of Biochem., Coll. of Agric., Univ. of Wisconsin, Madison, Wis.) Dietary fat and tumor formation. Cancer Research, 1:181-187. 1941.

When limited amounts of 20-methylcholanthrene were applied to the skin of mice receiving a control diet, tumors developed in only 12% of the animals. The addition of fat to the diet increased tumor formation to 83%. The tumor-promoting activity of the fat was found to reside in the fatty acid fraction. Ethyl laurate was as effective as natural glycerides; glycerol and the unsaponifiable fraction had only slight activity. The action of fat was increased by heating at 300° C. for 1 hour. Rancidification with ultraviolet light, or oxygenation in the presence of copper oleate failed to alter the effectiveness of the fat for tumor formation. The highest incidence of tumors appeared when fat was given throughout the experiment, but measurable increases were also observed when fat was fed either during the 1st 2 months while the carcinogen was applied, or after the 2nd month; *e.g.*, after the application of hydrocarbon had ceased. The most effective period was 1½ to 3 months after the beginning of the application of hydrocarbon. Dietary fat was much less effective in promoting induced skin tumors in the rat than in the mouse. Oil applied locally increased the rate of tumor formation in rats. It is suggested that the difference between the 2 species may be due to differences in skin thickness, in the rates of destruction of the hydrocarbon, and in the ability of the 2 species to metabolize fat.—Authors' summary.

TENNANT, R., A. A. LIEBOW, and K. G. STERN. (Depts. of Pathol. and Physiol. Chem., Yale Univ. Sch. of Med., New Haven, Conn.) Effect of macromolecular material from chick embryos on growth rate of mouse heart fibroblast cultures. Proc. Soc. Exper. Biol. and Med., 46:18-21. 1940.

A macromolecular fraction isolated from chick embryo extracts by differential high-speed centrifugation essentially according to Claude, exerts a distinct growth-stimulating effect on cultures of mouse heart fibroblasts.—Authors' summary.

SHARPLESS, G. R. (Dept. of Laboratories, Henry Ford Hosp., Detroit, Mich.) Choline and epithelial hyperplasia in the forestomach of rats. Proc. Soc. Exper. Biol. & Med., 45: 487-488. 1940.

Rats fed a basal diet lacking choline developed hyperplasia of the epithelium of the forestomach. The addition of 0.15% choline hydrochloride to the basal diet prevented the development of these lesions.—S. B-J.

STEKOL, J. A. (Dept. of Chem., Fordham Univ., New York, N. Y.) Conversion of dibenzyl disulfide to hippuric acid in the rat. Proc. Soc. Exper. Biol. & Med., 45:693-695. 1940.

Dibenzyl disulfide was fed to rats and hippuric acid was isolated from the urine and identified by analysis. The results support the suggestion that benzyl mercaptan or dibenzyl disulfide may be formed *in vivo* from S-benzyl-d-cysteine *via* S-benzyl-thio-

pyruvic acid. Certain phases of the metabolic transformation of sulfhydryl and cysteine derivatives of certain carcinogenic compounds are discussed.—Author's summary.

Immunology

BRYAN, W. R. (Nat. Cancer Inst., Bethesda, Md.), D. W. BEARD, and J. W. BEARD (Duke Univ., Sch. of Med., Durham, N. C.). The complement-fixing capacity of the rabbit-papilloma-virus protein. J. Nat. Cancer Inst., 1:197-203. 1940.

The mean quantity of purified papilloma protein necessary to bind 1 unit of complement was $0.95 \pm 0.06\ \gamma$, or $10^{-6.023}$ gm., with a coefficient of variation in the individual estimates of $\pm 29.8\%$. In previous work, the infectious unit (defined as that amount of protein injected under standard conditions necessary to produce papillomas at 50% of the injection sites), was found to be $10^{-8.355}$ gm. Thus the ratio of the complement-fixing unit to the infectious unit as far as their protein content was concerned was 215:1. The authors believe that the complement-fixation test may be useful not only in assaying the proteins of such materials but also in indirectly measuring their infectivity.—L. L. W.

Leukemia

DOLJANSKI, L., and M. PIKOVSKI. (Cancer Labs., Dept. of Exper. Path., The Hebrew Univ., Jerusalem.) Cultures in vitro of blood cells, bone marrow, and myocardium from leukotic fowls. Cancer Research, 1:205-216. 1941.

The progressive development of cultures of leukotic blood cells, leukotic bone marrow, and heart muscles from leukotic chickens is described. The fowls used were inoculated with Engelbreth-Holm's strain T of chicken hemocytoblastosis. Leukosis and sarcoma were produced by this agent. The agent remained virulent in tissue cultures as long as 181 days, through several transfers. In the authors' opinion fibroblasts developed from leukotic blood cells and "the agent of fowl leukosis is capable of remaining active and of increasing in the presence of nonspecific mesenchymal cells (fibroblasts)." The paper includes 15 photomicrographs.—S. B-J.

KIRSCHBAUM, A., L. C. STRONG, and W. U. GARDNER. (Dept. of Anat., Yale Univ. Sch. of Med., New Haven, Conn.) Influence of methylcholanthrene on age incidence of leukemia in several strains of mice. Proc. Soc. Exper. Biol. & Med., 45:287-289. 1940.

Mice of the inbred F strain which shows a high incidence of spontaneous leukemia were painted with methylcholanthrene twice weekly according to the technic of Morton and Mider. Mice of the C3H strain received similar treatment. Other nonleukemic strains (NH, CHI, CBAN, and C57) were painted once a week. Painting with methylcholanthrene was begun when the mice were about 30 days old. Details are presented in a table. F mice painted with methylcholanthrene developed leukemia at an earlier age than untreated controls of this strain. Myelogenous leukemia, which does not appear in untreated F mice before 300 days of age, appeared as early as 97 days after birth in treated mice. Only 3 cases of leukemia occurred among 184 mice of nonleukemic strains painted with methylcholanthrene. The effectiveness of methylcholanthrene in influencing the appearance of leukemia in young mice depended on the genetic susceptibility of mice to the disease—S. B-J.

Transplantation

BLUMENTHAL, H. T. The effects of spontaneous and transplanted tumors on the red and white cells in circulating blood and bone marrow. Cancer Research, 1:196-204. 1941.

The leucocytic response in the peripheral blood to transplanted normal tissues which had previously been shown to parallel closely the changes occurring locally about transplanted tissues, as described by Loeb, was used to determine the presence of the organismal differentials in tumors. Pieces from various kinds of rat and mouse tumors were homoio- and heterotransplanted into rats, mice, and guinea pigs; benign as well as malignant tumors were used. They all exerted the same effects on the leucocytes in the circulating blood as did normal tissues, the degree and kind of changes depending mainly upon the relation of the organismal differentials of host and transplant. However, benign tumors reacted with a slightly diminished intensity. Successive transplantations of tumor pieces likewise acted like those of normal tissues, and tumors and normal tissues interacted in the same way as did 2 normal or 2 tumor pieces successively transplanted; that is, a 1st homiotransplantation of either tumor or normal tissue caused an accelerated leucocytic response to a 2nd transplantation of either tumor or normal tissue. However, when tumor homiotransplants reached a large size the animals became progressively debilitated, developed an anemia and a leucocytosis, with a secondary hyperplasia of red and white cell elements of the bone marrow. This leucocytosis must be distinguished from the increase in polymorphonuclear leucocytes caused by the transplantation of heterogenous normal or tumor pieces; it is a condition which was not found after homiotransplantation of normal tissues. The anemia and accompanying leucocytosis are the only significant differences in the reactions of the host to normal and to tumor tissues, and this particular reaction to tumors is not connected with the organismal differentials of the tissues.—Author's abstract.

LUCKÉ, B., and H. SCHLUMBERGER. (Lab. of Path., Sch. of Med., Univ. of Pennsylvania, and The Morris Biol. Farm of the Wistar Inst., Philadelphia, Penn.) The effect of temperature on the growth of frog carcinoma. I. Direct microscopic observations on living intraocular transplants. J. Exper. Med., 72:321-330. 1940.

The adenocarcinoma of the leopard frog, when implanted into the anterior chamber of the eye, offers excellent material for the study of the influence of temperature on the growth of neoplasms. The transplants can be observed directly. Changes due to differences in temperature can be studied over a wider range in the frog than in warm-blooded animals since the body temperature is practically that of its surroundings. In this work, the temperatures to which the inoculated frogs were exposed ranged from 4 to 28° C., these being the extremes at which frogs could be maintained satisfactorily. Under these conditions, the temperature within the eye corresponded to that of the environment, as ascertained by thermocouple measurements. Regardless of temperature, frog carcinoma in the eye has a definite cycle of growth. After a period of lag comes a relatively rapid increase in size, followed by a slowing down of the rate of growth, then a stationary period and finally regression. The most important difference observed was an increase in the rate of growth at higher temperatures, and retardation at lower. At the higher temperature, there was earlier and more effective vascularization, the tumors forming long, branching, tubular outgrowths and cysts. At lower temperatures, the outgrowths were short and straight and cysts were uncommon. These differences were accentuated by repeated passages in the eye. Growth was checked during hibernation at 4° C. for 80 days, but no injury to the tumor resulted from this long exposure at low temperature and growth was resumed when the frogs were brought to higher temperatures. The paper is illustrated by 28 photographs.—A. C.

LUCKÉ, B., and H. SCHLUMBERGER (Lab. of Path. Sch. of Med., Univ. of Pennsylvania and The Morris Biol. Farm of the Wistar Inst., Philadelphia, Penn.) Heterotransplantation of frog carcinoma: Character of growth in the eyes of alien species. J. Exper. Med., 72:311-320. 1940.

Previous work by the authors has shown that the kidney carcinoma of the leopard frog can grow readily when transplanted

into the anterior chamber of the eye of animals of the same species. The method permits direct observation of the growth with the naked eye or under the microscope. The present paper deals with an attempt to establish whether or not the frog carcinoma can proliferate in the eyes of alien hosts. Two species belonging to the same family (green frog and bullfrog), a species from a different family (toad), and 2 different classes of cold-blooded vertebrates (gold fish and alligator) were used. The tumor was found to grow as readily in the eyes of frogs of alien species as in the eye of the natural host. In toads, the tumor grew well but the proportion of successful transplantations was distinctly less (37% as compared to 61 to 67% in the frogs). In frogs and toads, the character and rate of growth of the transplants was practically the same. The neoplastic cells retained their acinar arrangement, supported by a stroma which developed as well from the tissues of alien hosts as from those of the natural host. No progressive growth occurred in the fish, although characteristic acini persisted and a few mitoses could be found long after implantation. In the alligator, no growth occurred and the transplant deteriorated rapidly. No reaction developed in the eyes of any of the amphibians whereas a marked inflammatory reaction did occur in the eyes of fish and reptiles. The results indicate that, among cold-blooded vertebrates, the humors and tissues of the eye have a high degree of tolerance for foreign tumor grafts and that the success of transplantation into alien species decreases as the relationship to the original species becomes more distant. Seventeen photographs illustrate the paper.—A. C.

Reviews

LOEB, L. (Lab. of Research Path., Oscar Johnson Inst., Washington Univ. Sch. of Med., St. Louis, Mo.) The significance of hormones in the origin of cancer. J. Nat. Cancer Inst., 1:169-195. 1940.

This comprehensive review, covering the period from 1916 to 1940, presents summaries, discussions, and interpretations of investigations of the role of hormones of the ovary, adrenal, and pituitary glands in the production of cancer. While most of the material presented deals with the relation of hormones to carcinomas of the mammary gland and genital tract of mice and rats, other species and other types of tumors are included, and some features of cancer of the breast in women are discussed. Genetic factors and the possible role of viruses are analyzed. After discussing both rhythmic and noncyclic growth processes the author concludes that "the study of the origin of cancer is, at the same time, a study of the mechanisms which in the normal organism tend to prevent the development of these abnormal growth processes." The bibliography contains 153 references.—S. B-J.

CLINICAL AND PATHOLOGICAL REPORTS

BRAGA, A. (Lisbon, Portugal.) A fotografia com Radiaçoes infra-vermelhas aplicada aos Tumores. Infra-red photography applied to tumors. Arq. de pat., 12:160-171. 1940.

Ilford film, artificial light, and radiations of from 7,000 to 9,000 Å were used. Twelve sets of ordinary photographs compared to infra-red photographs are included.—M. D-R.

PERRÍN, T. G. (Fac. Med., Mexico, D. F.) Sobre el diagnóstico histopatológico en el curso de las intervenciones quirúrgicas. Concerning histopathological diagnosis in the course of surgical operations. An. méd., 1:7-12. 1940.

The author reviews the recent literature on the subject and summarizes his own method used successfully for 16 years. Pieces of tissue less than 0.5 cm. thick are fixed 1 minute in boiling 15% formaldehyde solution. This is followed by sectioning in the freezing microtome, staining of the sections on a slide with 0.5% toluidine blue for 20 seconds, rapid washing with water, and mounting and observation in neutral glycerol. Four photomicrographs are appended.—M. D-R.

TENOPYR, J., and I. SILVERMAN. (Brooklyn, N. Y.) The importance of biopsy in tumor diagnosis: A report of experience with a new biopsy needle. Roentgenology, 36: 57-60. 1941.

This is a further report on the use of a new biopsy needle devised and previously described by the junior author.—E. A. L.

Radiation—Diagnosis and Therapy

BOGART, F. B. (Chattanooga, Tenn.) Irradiation treatment of cancer of the skin. Radiology, 36:12-22. 1941.

Various methods of irradiation of carcinomas of the skin are presented and illustrated with case reports. Massive doses of medium or low voltage x-rays with little or no filtration are used for practically all small lesions. High voltage, heavily filtered x-rays in fractionated doses are preferred for the large indurated lesions, all lesions involving cartilage, and all carcinomas of the lip. In selected cases x-ray treatment is supplemented with interstitial low intensity radium element needles.—E. A. L.

CHONT, L. K. (Univ. of Oklahoma Hosp., Oklahoma City, Okla.) Sarcomas of the small intestine and reference to their radiosensitivity. Radiology, 36:86-97. 1941.

One case of leiomyosarcoma and 3 of lymphosarcoma of the small intestine are reported, and the literature is reviewed. Thirty-four cases of leiomyosarcoma of the small intestine and over 400 of lymphosarcoma have been recorded in the literature. The author's case of leiomyosarcoma was the only one to have been irradiated. It responded well. Lymphosarcomas are radiosensitive.—E. A. L.

FRIEDMAN, M. (Bellevue Hosp., New York, N. Y.) The treatment of carcinoma of the corpus uteri: Description of a new hysterostat. Radiology, 35:28-35. 1940.

A new radium applicator, called a hysterostat, for the intracavitary radiation of carcinoma of the corpus uteri is described. It consists of a central crosspiece and 2 to 4 lateral tandum inserters. The central crosspiece is attached to a stem permitting variation of the angle between them. The lateral inserters are not fixed instruments but are separate pieces which can be interchanged to make various shapes to correspond to the shape of the uterine cavity.

Eight of the 13 patients treated with this instrument were subjected to total hysterectomy 4 to 6 weeks after the radium treatment. Seven of the 8 showed no residual cancer. Three of the remaining 5 are alive and well 6 to 24 months after treatment. The dose varied between 4,000 and 7,660 mgh.—E. A. L.

HAWLEY, S. J. (Geisinger Memorial Hosp., Danville, Penn.) Rotation therapy. Radiology, 35:65-69. 1940.

A method is described for rotation x-ray therapy in which the beam is directed horizontally towards the tumor area and the patient is rotated on a vertical axis. The advantage of this method is that there is a volume of tissue surrounding the axis of rotation which is continuously in the beam and receives the most intense radiation, whereas the skin receives the least radiation owing to the fact that it is moving most rapidly and is in the narrowest portion of the beam.—E. A. L.

LAWRENCE, J. H. (Univ. of California, Berkeley, Calif.) Nuclear physics and therapy: Preliminary report on a new method for the treatment of leukemia and polycythemia. Radiology, 35:51-59. 1940.

The use of radioactive isotopes as tracers and as substitutes for radium in direct radium therapy is discussed. Four representative cases of chronic myelogenous leukemia and 1 of chronic

lymphatic leukemia treated orally with radiophosphorus in the form of sodium phosphate are reported. The responses were similar to those usually following x-ray or radium therapy. To avoid a cathartic effect the total dose of sodium phosphate was kept below 3 gm. The dosage of radiophosphorus has been determined by experimentation and is probably too small. No patient received more than the equivalent of 3 r daily whole body radiation.

Two cases of polycemia vera treated in the same manner are also presented.—E. A. L.

MURPHY, J. T., and C. E. HUFFORD. (Toledo, Ohio.) The use of 200,000 volts in the treatment of advanced superficial cancer. Radiology, 36:23-31. 1941.

Protracted fractionated x-ray irradiation with high voltage and heavy filtration is offered as a method of treating advanced carcinoma of the skin. Illustrated with case reports.—E. A. L.

O'BRIEN, F. W. (Boston City Hosp., Boston, Mass.) Radiation of cancer of the cervix. Radiology, 35:23-27. 1940.

A series of 289 patients seen from 1923 to 1935 inclusive is presented. Twenty-seven of the cases were judged unsuitable for any radiation therapy. Thirty-five per cent of the remaining 262 were in stages 1 and 2 and 65% in stages 3 and 4.

The development of radium therapy in the early years of the series is discussed. In 1930 x-ray therapy was instituted and has been used since then in conjunction with radium. In 1933 a follow-up clinic was established, and it has been possible to follow 28 patients for 5 or more years. There was a 5 year survival in 7 cases. Six of the 7 died with disease in the 6th year and 1 remained free of demonstrable disease.

The need for increasing the radiation dose to the parametria is emphasized.—E. A. L.

ROSH, R. (Bellevue Hosp., New York, N. Y.) Factors influencing the prognosis in the treatment of carcinoma of the cervix. Radiology, 35:17-22. 1940.

This is an analysis and discussion of the problems and complications encountered in treatment of carcinoma of the cervix. From 1925 to 1939, inclusive, 795 patients were seen. Until the last 4 or 5 years no case could be classed as stage I, the greater number being in stage IV. Two hundred sixty-one were transferred elsewhere for custodial care; 303 were given the complete course of treatment.

Successful treatment depends upon the general condition of the patient, stage of disease, quality of irradiation, continuity of treatment and individual response. Complications may arise in the skin from slow vascular changes; in the urinary tract from extension of the disease causing ureteral obstruction; in the rectum from small ulcerations; in the vagina from fistulae; and may arise from local extension of the disease causing intractable pain.—E. A. L.

WIGBY, P. E., and M. COHEN. (Parkland Hosp., Dallas, Texas.) Radiation therapy of carcinoma of the skin: An analysis of 83 lesions in 70 patients. Radiology, 35:70-78. 1940.

The paper presents methods of irradiating carcinoma of the skin previously reported by others and discusses the methods used by the authors. Superficial basal cell lesions should receive between 4,000 r and 5,000 r low voltage therapy. Daily doses between 600 r and 1,000 r are given. For the larger and thicker basal cell tumors and for squamous cell carcinomas, various combinations of medium and high voltage therapy are used. The total dose is between 5,500 r and 6,000 r, and about 200 r are given daily.—E. A. L.

Skin and Subcutaneous Tissues

ESCALONA, E. (National Univ., Mexico, D. F.) Angiomatosis de Lindeau y Von Hippel con adenomas sebaceos simétricos tipo Pringle. Angiomatosis of Lindeau and Von Hippel with symmetrical sebaceous adenomas of Pringle's type. Medicina, México, 20:205-209. 1940.

Report of 1 case.—M. D-R.

MILLAN GUTIERREZ, J. (Dept. Dermatol. General Hosp., Mexico, D. F.) Adenomas sebaceos simétricos. Symmetrical sebaceous adenomas. Medicina, México, 20:199-205. 1940.

A case belonging to the neuro-ectodermic dysplasias of Von Bogaert, the clinical variety being that of the sclerosis tuberosa of Bourneville Brissaud. Four illustrations are appended.—M. D-R.

Female Genital Tract

MASCIOTTRA, R. L., and A. A. DÍAZ COLODRERO. (Rivadavia Hosp., Buenos Aires.) El carcinoma de la glándula de Bartholino. Carcinoma of Bartholin's gland. Rev. med.-quir. de pat. fem., 14:889-908. 1939.

The extreme rarity of neoplasia in this location contrasting with the frequency of infection is emphasized, only 39 cases having so far been reported (Simendinger, E. A., Surg., Gynec. & Obst., **68**:952. 1939). Two cases are described, 1 of squamous cell carcinoma and the other of adenocarcinoma. No metastases were observed, and the tumors gave the impression of being cysts under pressure. Excision was carried out in both cases and recurrence of the squamous cell tumor occurred shortly after. The paper is illustrated with 14 photomicrographs.—M. D-R.

DIONISI, H. (Maternity Inst., Buenos Aires.) Tumor de la teca interna del ovario. (Fibroma Thecocellulare xanthomatodes ovarii de Löffler y Priesel.) Tumor of the inner theca of the ovary. Rev. méd.-quir. de pat. fem., 15:18-49. 1940.

This is a case of the hormone-secreting tumors first described in 1932 by Löffler and Priesel (Beitr. z. path. Anat. u. z. allg. Path., **90**:199. 1932). It originated in a woman 32 years old showing uterine hemorrhages and hyperplasia of the endometrium of a folliculinic origin. The tumor had the appearance of a fibroma with a tendency to epithelioid transformation. The cells were extremely rich in lipoids as shown by staining reactions and chemical and optical analysis. The paper includes 29 references and 22 pictures of gross and microscopic preparations.—M. D-R.

SCHILLER, W., and D. D. KOZOLL. (Cook County Hosp., Chicago, Ill.) Primary signet-ring cell carcinoma of the ovary—with a case report. Am. J. Obst. & Gynec., 41:70-78. 1941.

A case thought to be a primary signet ring cell carcinoma of the ovary (Krukenberg tumor) is reported. The status of this type of tumor, whether primary in the ovary, or metastatic from primary intestinal adenocarcinomas, is discussed. Histological characteristics differentiating the Krukenberg tumor from the pseudomucinous cystadenoma are considered.—A. K.

Gastrointestinal Tract

COLILLAS, D., and R. L. MASCIOTTRA. (Rivadavia Hosp., Buenos Aires.) Schwannoma del intestino delgado. Schwannoma of the small intestine. Rev. méd.-quir. de pat. fem., 15:245-250. 1940.

The authors summarize the characteristics whereby gastric schwannomas are recognized according to the recent work of Masson, Gosset, Roussy, etc. The are: (a) presence of whorls of fusiform pseudo-connective tissue cells showing anastomoses; (b) presence of cylinders of cells by isolation of some nodules of the whorls; (c) formation of palisades; and (d) microcystic degeneration of the cells. The tumors are slow-growing, benign, and do not affect the general condition of the patient. All of these clinical and histopathological traits were found in the reported case which would be the first example of a jejunum-

ileal schwannoma. The tumor was removed together with 20 cm. of intestine, and the patient, a woman of 44, promptly recovered. One color plate of the growth and 2 photomicrographs are appended.—M. D-R.

Bone

MANZANILLA, M. A. (Mexico, D. F.) Osteocondroblastoma metacarpal primitivo. Primary metacarpal osteochondroblastoma. Medicina, México, 20:18-31. 1940.

Report of 1 case accompanied by 4 illustrations.—M. D-R.

MORAIS, E. (Lab. pat. An., Fac. of Med., Porto, Portugal.) Tumores giganto-celulares. Giant cell tumors. Arq. de pat., 12:5-98. 1940.

A report based on the study of 28 giant cell tumors (6 in the tendon sheaths, 18 in the jaw bones, 3 in the large bones, and 1 in a rib) and also on that of the small lesions induced in 10 rabbits fed large amounts of cholesterol and in which tendons were experimentally injured. The primary lesion of all these pseudo-tumors is one consisting of hystiocytes. This lesion may later develop into a fibroblastic, xanthomatous, or giant cell growth. All these lesions have a remarkable tendency to become sclerotic, and their varied morphological aspects depend either on their type or on the phase of their evolution. Although hypercholesterinemia, associated or not with trauma, is a factor in the genesis of the lesions, the part it plays is only subsidiary. Various other factors such as bacterial toxins, endocrine disturbances, vitamin deficiency, circulatory alterations, *etc.*, acting either alone or in combination, are sufficient in most cases to explain the different reactions of the bone tissues. Among them one must place the so-called tumors of myeloplaxes or giant cell tumors. These granulomatous lesions are entirely different both clinically and histologically from the giant cell sarcoma, and the latter should not be segregated from the general sarcoma group no matter how rich in giant cells they are. The literature is thoroughly reviewed and about 250 references are appended. The text contains 32 illustrations.—M. D-R.

Leukemia, Lymphosarcoma, Hodgkin's Disease

de OLIVEIRA-CAMPOS, J. (Lisbon.) Leucémie myeloblastique a manifestation tumorale. Myeloblastic leukemia with tumor formation. Arq. de pat., 12:146-159. 1940.

The author describes a case diagnosed clinically and cytologically as myeloblastic leukemia where a tumor composed of myeloblasts destroyed the prostatic and periprostatic tissues. Seven photomicrographs and 30 references are appended.—M. D-R.

STEINER, P. E. (Dept. of Path., Univ. of Chicago, Chicago, Ill.) Reliability and significance of the Gordon test in Hodgkin's disease. Arch. Path., 31:1-10. 1941.

The Gordon reaction was positive in 16 (76.2%) of 21 cases of Hodgkin's lymphogranuloma studied by the author. Of 310 cases previously reported the test was positive in 229 (73.9%). The author found no positive reactions with material from lymph nodes from 40 nongranulomatous conditions. Of 452 control cases previously reported positive reactions were reported in 8 (1.7%). The Gordon reaction is considered reliable in differential diagnosis of lymph node diseases if it is accompanied by histological examination.

The Gordon agent, extracted from lymphogranulomatous tissue, causes spastic paralysis, incoordination, ataxia, retraction of the head, fits, and loss of weight in rabbits and guinea pigs after intracerebral injection. A photomicrograph is reproduced showing disappearance of Purkinje cells in the cerebellum following injection of the agent. The author concludes that the distribution of the Gordon agent is such as to make it unlikely that it is the causative agent of Hodgkin's disease. Its properties are those of a nonliving agent, probably enzymatic. The test, while reliable, is nonspecific.—S. B-J.

Statistics

APPERLY, F. L. (Dept. of Path., Med. Coll. of Virginia, Richmond, Va.) The relation of solar radiation to cancer mortality in North America. Cancer Research, 1:191-195. 1941.

The relationship between the incidence of skin cancer and general cancer rates is shown to be direct in cold climates and inverse in hot climates. The total cancer mortalities of the various North American states and Canadian provinces are shown to fall with increasing solar radiation and with the numbers of people exposed thereto and are independent of the production of skin cancer. A correlation is suggested between immunity to cancer and exposure to solar radiation or to artificial sources of ultraviolet light. Such exposure is proposed as a means of reducing mortality from cancer. The paper is illustrated by 5 graphs summarizing statistics of cancer mortalities among persons exposed to solar radiation in the American states and Canadian provinces and the relation of cancer mortality rates to Smith's solar radiation index in various regions.—S. B-J.

MACKLIN, M. T. (Univ. of Western Ontario, London, Canada.) An analysis of tumors in monozygous and dizygous twins. J. Hered., 31:277-290. 1940.

A review of the literature with 15 new cases not previously published. Both members of monozygous twins have tumors more frequently, have the same type in the same organ and an age of onset more nearly identical than do both members of dizygous twins. Heredity plays an important role in the production of tumors and the age at which they develop.—J. J. B.

Cancer Control and Public Health

MacFARLANE, C., F. S. FETTERMAN, and M. C. STURGIS. (Woman's Med. Coll. of Pennsylvania, Philadelphia, Penn.) An experiment in cancer control. Preliminary report on periodic pelvic examinations of one thousand well women. Am. J. Obst. & Gynec., 39:983-989. 1940.

It is stated that the percentage of uterine cancer cases which are cured varies inversely with the stage of the disease in which the patient receives treatment. This statement is supported by the data from 50 cases of cancer of the cervix. Periodic pelvic examinations of 1,000 women 30 years of age and over are being made by the authors. This report covers the 1st and 2nd examinations. In the 1st examination 4 uterine malignancies were found, 3 of the cervix and 1 of the corpus. Benign lesions (papillomas, leucoplacic areas, polyps, endocervicitis, myomas, *etc.*) were found in 318 women, treatment being carried out in 113 cases. After an interval of 6 months a 2nd examination was made. No malignancies were found. Old benign lesions were present in 177 women, new lesions were found in 69 women. Of the 4 patients with malignant lesions 2 might have waited weeks or months before reporting to their physicians, since there were no pelvic symptoms except a moderate amount of leucorrheal discharge. The opinion is expressed that areas of chronic epithelial irritation predispose to the development of cancer and the only way to discover early cervical lesions is by means of periodic pelvic examinations.—A. K.